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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 6.

BIOCHEMICAL ANALYSES WITH THE SPEKKER ABSORPTIOMETER.

I. Estimation of Urea, Citrulline, Allantoin and Related Carbamido Compounds.

By

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[Read June 5, 1945. Published separately May 10, 1946].

The carbamido-diacetyl reaction described by Fearon (1939) has been used for the direct colorimetric estimation of citrulline by Gornall and Hunter (1941) and Archibald (1944), and for the estimation of urea by Abelin (1940), Ormsby (1942), Barker (1944), and Archibald (1945). No fundamental alteration was made by these workers, although each one adopted a different procedure. During an extended study of the test in this laboratory, the optimum conditions for the reaction were determined with the Spekker photoelectric absorptiometer (Adam Hilger & Co., Ltd.), and calibration curves for urea, methyl urea, citrulline, and allantoin were constructed.

The test essentially consists in boiling the substrate with strong acid and diacetyl, and intensifying the resulting pigment by a suitable oxidiser. With urea, a yellow pigment develops; with a mono-substituted urea, the pigment is red. The urea pigment in low concentration has a green tinge; the red substituted urea pigment in high concentration has a yellow component that causes the pigment to appear almost orange. Maximum absorption for the urea colour is at 475 mμ; for the red pigment it is between 485-495 mμ (Barker, 1944). In order to obtain maximum colour intensity, the pigment must be oxidised; but if over oxidised the pigment rapidly fades. With certain precautions satisfactory results are obtained by addition of the minimum necessary amount of 1 per cent potassium persulphate, a method which was originally used by Fearon, and has been adopted by subsequent workers.

For the purpose of quantitative estimation the test has two objections. Firstly, and most important, persulphate oxidation is difficult to control. Using only traces of persulphate, bleaching rapidly overtakes maximum colour formation; the red pigment especially is sensitive to the hydrogen peroxide liberated from the persulphate. Abelin (1940) overcomes the difficulty by using no oxidiser at all. Fearon found arsenic acid a suitable oxidiser (personal communication). The acid acts only in the boiling mixture and ceases to oxidise vigorously when the solution is cooled to room temperature. Phosphoric acid acts in a similar way. It was introduced into the test by Archibald (1945). This simplifies the procedure and lessens the oxidation hazard.

A second objection is raised by Ormsby (1942), who states that every time a new batch of diacetyl is used a new curve has to be constructed. Using diacetyl monoxime as a source of diacetyl it is found that in 3 per cent. concentration the

reagent tends to precipitate and form a brown polymer, thereby altering in strength. These difficulties have been overcome, and a diacetyl reagent is obtainable that gives identical curves with different batches of diacetyl monoxime. Archibald (1945) uses α-isonitrosopropiophenone instead of diacetyl monoxime, and claims that it is a more specific reagent for urea ; however, diacetyl monoxime is a much more sensitive reagent than α-isonitrosopropiophenone and is preferred by the present author. Satisfactory results can be obtained with either reagent.

Outline of Method.

Apparatus. A Spekker photoelectric absorptiometer, or any other type of absorptiometer may be used. The fused quartz cups supplied with the Spekker instrument are unsuitable for immersion in a water-bath. The pigment is generated in 9 ml. long-stem Folin-Wu tubes (as used for blood-sugar estimations). The long stems conveniently act as condensers. A metal rack can be constructed to hold six tubes upright in a one litre Pyrex beaker. The edge of the lower plate of the rack is turned down so as to raise it a sufficient distance to prevent the tubes making contact with the floor of the beaker. As an alternative, an electric immersion heater in a litre beaker can be used ; but care must be taken that the tubes make no contact with the metal of the heater.

Colour Filters. The spectrum blue gelatine filter (No. 602) with a maximum transmission close to 475 mμ is used for the yellow pigment (urea, allantoin). The blue-green gelatine filter (No. 603) with a maximum transmission at 500 mμ is used for the red pigment (citrulline, methyl urea). If no gelatine filters are available glass filter No. 7 should be used for both types of pigment.

Reagents. The following oxidising acid mixtures give satisfactory results :—

(*A*) One volume of concentrated sulphuric acid is added to three volumes of syrupy phosphoric acid (Sp.gr., 1·75). Any turbidity in the mixture settles out on standing, and the clear supernatant liquid can be poured off for use.

(*B*) 50 ml. of concentrated sulphuric acid are added to 50 ml. of 20 per cent. arsenic acid.

(*C*) Ten grams of arsenic acid, or the molecular equivalent of sodium arsenate are dissolved in 100 ml. of concentrated (36 per cent.) hydrochloric acid.

The following diacetyl reagent keeps satisfactorily for at least two months at room temperature and shows no discolouration or precipitate. New batches of diacetyl reagent have given identical results. Dissolve one gram of diacetyl monoxime in 100 ml. of 5 per cent. acetic acid. If the diacetyl monoxime is not perfectly white, the solution can be decolourised with norite charcoal.

Standard Solutions. Only pure analytical reagents were used. The citrulline was prepared according to the method of Fox (1938). The substances are dried before weighing ; volumetric solutions containing 30 mg. are made up with glass-distilled water ; a few drops of toluene are added as preservative. From these stock solutions dilutions of 1:10 and of 1:2 were prepared. The 1:10 dilution contains 15 μg in 0.5 ml. ; the 1:2 dilution contains 75 μg in 0.5 ml.

General Considerations. Both types of pigment do not obey the Beer-Lambert absorption laws, therefore a graph where concentration is plotted against density shows a curve. The only part of the curve for which one could substitute a straight line without involving an error greater than 1 per cent. is that from 30 μg to 90 μg

in the case of urea and methyl urea, and from 75 μg to 180 μg for citrulline. Values below or above these ranges lie on definite curves, bent in opposite directions. It is essential therefore to construct an accurate curve. At least 8 points should be determined ; from 0 μg to 90 μg they should be taken at 15 μg intervals, and from 90 μg to 150 μg at 30 μg intervals.

The procedure now is as follows : Measure into the Folin-Wu tube the amount of standard solution necessary to yield one of the pre-determined points on the curve, now add 2 ml. of the diacetyl reagent and either 3 ml. of acid mixture (*A*) or 4 ml. of acid mixtures (*B*) or (*C*). The volume is made up to the 9 ml. mark with distilled water. The tube is stoppered and inverted to mix the contents thoroughly, and then put into a vigorously boiling waterbath.

The boiling is maintained for 20 minutes, if urea or allantoin is estimated, but only for 15 minutes in the case of citrulline and methyl urea. If the level of the water bath reaches just to the 9 ml. mark the upper part of the tubes remain sufficiently cool to act as efficient condensers, and there is no need to adjust the volume after boiling.

After removal from the waterbath, the tubes are put into running cold water for approximately 2 minutes, wiped dry, and poured into the comparator cups. If these cups are wet they can be rinsed with a small amount of the fluid from the Folin-Wu tubes, since the former only hold 8 ml. The colour intensity is measured at once with the Spekker absorptiometer, using the appropriate filters. No fading of the colour occurs for at least 30 minutes. The standard values obtained for urea, methyl urea, citrulline, and allantoin are given in Table 1.

Variable Test Conditions. The method just described was adopted after every reactant in the test was carefully checked. Any variation in the composition of the reagents, or in the test method leads to different results. The method for optimum colour production therefore could only be determined after varying each individual component of the test. Some of the results are grouped together in Table 2.

The importance of using the correct amount of diacetyl is outstanding. Slight errors in the amounts of diacetyl give rise to large errors in colour values. Using excess of diacetyl avoids this hazard. Acid concentration and boiling time are two other important factors. The optimum acid concentration was found to be 4 ml. for the hydrochloric acid mixture, and somewhat less than 4 ml. for the other acids. An increase beyond 4 ml. results in somewhat reduced colour values, especially when the substrate is in high concentration. Table 2 shows that optimum colour production has not been reached after boiling for 20 minutes, but the proportional increase in colour value after this time was not thought sufficient to lengthen the period of boiling as a routine measure.

Archibald (1944) recommends that the procedure should be carried out in the dark, since both types of pigment after development are photosensitive. On exposure to light the colour intensity gradually increases. In the present author's experience this does not occur at all when acid mixture (*C*) is used (see Table 2a) and is only very slight with acid mixture (*A*) after allowing the solutions to stand for 48 hours ; but for the practical conduct of the test this is of no importance. Archibald's observation is probably due to the failure to use excess of diacetyl in the test.

TABLE 1.

Density Values.

μg	Urea Acid (*A*)	Urea Acid (*C*)	Allantoin Acid (*A*)	Methyl Urea Acid (*C*)	Citrulline Acid (*C*)
15	0·07	0·07	<0·01	0·04	0·01
30	0·22	0·20	<0·01	0·13	0·05
45	—	0·36	<0·01	0·28	0·098
60	0·62	0·51	0·01	0·42	0·14
75	0·84	0·67	<0·02	0·57	0·21
90	1·02	0·80	0·02	0·70	0·28
105	1·24	0·94	—	0·82	0·35
120	1·31	1·05	—	0·92	0·42
135	—	1·15	—	1·01	0·49
150	1·55	1·26	—	1·08	0·55
165	—	1·34	—	1·15	0·62
180	1·69	1·40	—	1·20	0·68
210	1·75	1·50	—	1·30	0·80
240	1·78	1·56	—	1·38	0·90
270	—	1·60	—	1·44	0·98

TABLE 2*.

I. Varying the amount of diacetyl for 30 μg Urea Acid Mixture (*A*).

ml. of 1% diacetyl	0·2	0·6	0·8	1·0	1·2	1·4	1·8	2·0	2·4
Density Value	0·08	0·30	0·36	0·42	0·46	0·48	0·50	0·51	0·52

II. Varying the amount of HCl for 55 μg Urea. Same amount of oxidiser.

ml. conc. HCl	1	2	3	4	5
Density Value	0·28	0·37	0·45	0·46	0·43

III. Varying the time of boiling for 150 μg Urea. Acid Mixture (*C*).

Time in minutes	5	10	15	20	25
Density Value	0·61	1·02	1·16	1·26	1·28

* Unless otherwise stated the test conditions are as described for the standard method.

TABLE 2a.

Determination of Fading-Time for Colours developed by the Standard Method. Acid Mixture (*C*).

Time in Minutes	0	20	40	60	120
Density Value (Citrulline)	0·26	0·26	0·25	0·25	0·24
Density Value (Urea)	0·19	0·19	0·18	0·18	0·17
Density Value (Urea)	1·25	1·25	1·22	1·22	1·20

APPLICATION OF METHOD TO BLOOD.

The method is suitable for routine blood urea analyses. A micro or macro procedure can be adopted depending on the amount of blood available. To precipitate blood protein the tungstic acid method of Folin-Wu should be used. Cadmium or zinc protein precipitates, especially if alkaline, give too low and erratic values. A similar observation was made by Barker (1944). An outline of the procedure for 0·2 ml. blood is now given :—

Measure 3·4 ml. of distilled water into a small test-tube, and add 0·2 ml. of blood from a micro-pipette. The pipette is rinsed with the clear supernatant water before laking begins. The tube is shaken and set aside for a few minutes to allow laking to take place ; then 0·2 ml. of 10 per cent. sodium tungstate solution is added, followed by 0·2 ml. of $\frac{2}{3}$ N sulphuric acid. The tube is shaken again and set aside until the precipitate has turned brown. The contents of the tube are now poured on a small filter-paper (7 cm.). Two ml. of water-clear filtrate are measured accurately into a Folin-Wu tube (graduated at the 9 ml. mark), and in succession 2 ml. of diacetyl reagent and 3 ml. of acid mixture (*A*) are added. The tube is then filled to the 9 ml. mark with distilled water, inverted to mix all contents thoroughly, and immersed in a boiling water-bath for 20 minutes. After boiling the tube is cooled under running cold water to room temperature. The coloured solution is now poured into one of the Spekker absorptiometer cups. Using both the heat-absorbing filter and the gelatine filter No. 602 in the path of the light, the density value is measured on the instrument.

Calculation. With the aid of the standard density values given in the first column of Table 1, a curve is plotted, and the density value of the unknown solution (blood) is referred to this curve to determine its concentration.

The values on the graph read in μg present in 9 ml. volume, and from this the original blood concentration is calculated. Since the 2 ml. of filtrate used correspond to 0·1 ml. of whole blood, the μg value read off on the graph must be multiplied by 1000 to convert it to mg. per 100 ml. of blood. It follows that under these circumstances the value of μg on the graph can be read as mg. per 100 ml. of the blood samples, calculation formulae being thus avoided. If different amounts

of blood are used such a direct reading is not possible and the correct multiplication factors must be used.

The accuracy of the method is good when compared with the gasometric urease method. Archibald found the values to be 0·0 to 0·8 mg. per 100 ml. higher than those obtained with the urease method. Using urease and the diffusion technique for ammonia estimation (Kawerau, 1941), a few parallel estimations were carried out in this laboratory. The results are offered without comment in Table 3.

TABLE 3.

No.	Specimen	mg. urea per 100 ml. Colorimetric Method	Urease Method
1	Whole blood	24·0	22·0
2	Whole blood	114·0	112·0
3	Blood serum	68·0	69·5
4	Blood serum	62·2	61·5
5	Whole blood	60·0	60·0
6	Blood serum	57·0	54·8
7	Blood serum	96·0	97·6

The blood components that might give rise to an "extra-urea" value are citrulline, allantoin, and possibly arginine. Using 30 μg of each substance the relative interference values were estimated by the standard method. The results are given in Table 4.

TABLE 4.

Substance	Density Value of 30 μg Acid (*A*)	Acid (*C*)
Urea	0·22	0·20
Citrulline	0·05	0·03
Allantoin	0·01	0·02
Arginine	0·00	0·00

Archibald (1944) claims that the amount of citrulline in fasting normal human blood is between 0·3 and 1·0 mg. per 100 ml. of blood; and he gives a similar concentration range for what he terms "apparent allantoin". In using 0·2 ml. of blood in the analysis therefore only one third to one tenth of the amount used for the experiments in Table 4 is present, and from the observed density values it can be seen that these substances are not likely to interfere with the accuracy of the method.

Application of Method to Urine.

To carry out the reaction on urine, the urine must be free from pathological protein. The urine is adjusted to pH 4-5 with 2 drops of 2 per cent acetic acid, boiled and filtered. The filtrate when cold is diluted 1:100. Measure accurately 0·5 ml. of the diluted sample into a Folin-Wu tube and proceed by the method described for blood.

Although acid mixture (*A*) may be convenient for most workers, in this laboratory for both blood and urine analysis acid mixture (*C*) is preferred because in the case of urine it is less likely to give rise to extraneous colours. This is probably due to the fact that when finally set up for boiling the acid concentration in the Folin-Wu tube using mixture (*C*) is only half of that of mixture (*A*). Archibald (1945) found that with mixture (*A*) urea values averaged 2·8 per cent. higher than the concentrations indicated by the urease method.

Application of Method to the Estimation of Allantoin and Citrulline.

It is obvious from the foregone considerations that it is impossible to estimate allantoin and citrulline directly by this method. Special adsorbents for allantoin as well as for citrulline must be used in order to concentrate these substances before the general procedure is carried out. Details have been given by Archibald (1944), and the reader should consult the original paper.

If exactly identical procedures are adopted it has been found possible, in this laboratory, to estimate citrulline by using a methyl urea curve for reference. Gornall and Hunter (1941) point out that the colour intensity is not proportional for the two substances when allowance is made for their different molecular weights. Citrulline is 2·36 times the weight of methyl urea, and if true proportionality existed one could read the μg value of a citrulline pigment on a methyl urea curve and obtain the correct result by multiplying it by 2·36. Since no true proportionality exists on a molecular basis, the factor cannot be used. Still, it must be pointed out that the two curves run parallel to each other, and if the molecular factor does not fulfil the purpose, some empirical factor might be satisfactory. It has been found that the factor of 2 best suits this purpose. A methyl urea curve in which the μg values are multiplied by 2 can serve any citrulline estimation. The error involved in this transaction has been found to be not greater than ± 3 μg for any single point on the curve.

SUMMARY.

A simple direct colorimetric method for the estimation of urea and related carbamido compounds is described. The method is based on the carbamido-diacetyl reaction of Fearon, and has been modified for the purpose of quantitative assay.

The Spekker photoelectric absorptiometer is used to carry out the estimations. A method is described by which it is possible to use methyl urea as a standard for the estimation of citrulline.

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No. 7.

THE POSSIBILITY OF INITIATING THERMO-NUCLEAR REACTIONS UNDER TERRESTRIAL CONDITIONS.

By J. H. J. POOLE, Sc.D.

[Read November 27, 1945. Published separately May 6, 1946].

The recent developments in the generation of energy by uranium fission, have shown that it is possible to generate excessively high temperatures in masses of the order of 1 to 10 kg. It is quite simple to estimate the maximum temperature which could be attained by the complete fission of uranium assuming that all the energy was conserved. The energy per fission is about 177×10^6 electron volts or about $2{\cdot}8 \times 10^{-4}$ erg. (1). The temperature attained will be so high that we may assume that the fission nuclei are completely stripped of electrons, so that the fission energy will be divided equally between two fission nuclei and 92 electrons, a total of 94 particles. The small number of neutrons emitted may be neglected, as the figure I have quoted is for the energy of the fission products alone, and the total energy per fission is probably of the order of 200×10^6 e.v., so we shall arrive at an underestimate in any case. The mean thermal energy of an elementary particle at a temperature θ is $\frac{3}{2} k\theta$, where $k = 1{\cdot}38 \times 10^{-16}$ erg per degree ; and the energy of each of the 94 particles in the uranium fission is $2{\cdot}8 \times 10^{-4}/94$ or 3×10^{-6} erg. Hence their temperature is $3 \times 10^{-6}/2 \times 10^{-16}$ or $15{,}000 \times 10^6$ degrees. Since at the centre of the sun, whose energy is supposed to be supplied by thermo-nuclear reactions, the temperature is probably of the order of 20×10^6 degrees, it is obvious that the initiation of such reactions by uranium fission is worth considering.

The largest known source of thermo-nuclear energy on the earth is probably the ocean. Solar energy is believed to be generated indirectly by the formation of He^4 from four protons. In this process for every helium nucleus formed $4{\cdot}2 \times 10^{-5}$ erg is generated. Now the total mass of hydrogen in the ocean is about $1{\cdot}6 \times 10^{23}$ g. (2), and since the number of protons per gram of hydrogen is 6×10^{23}, the total number of protons in the ocean is $9{\cdot}6 \times 10^{46}$. Hence the total energy which could be obtained from the ocean by this method is $\frac{1}{4}[9{\cdot}6 \times 10^{46} \times 4{\cdot}2 \times 10^{-5}] = 10^{42}$ ergs.

The amount of hydrogen in the lithosphere would probably only increase this figure by about 10%, and hence need not be considered.

It is of some interest to estimate to what temperature this quantity of energy, if suddenly generated, would raise the temperature of the earth. The mass of the earth is 6×10^{26} g., hence the energy available per gram of earth is $10^{42}/6 \times 10^{26}$

$= 1{\cdot}67 \times 10^{14}$ ergs. Now Eddington has shown that for a mixture of any set of elements at very high temperatures, the mean atomic weight may be taken as 2, provided no large amount of hydrogen is present. Thus the number of particles between which the $1{\cdot}67 \times 10^{14}$ ergs is to be divided will be 3×10^{23}. Hence the energy of each particle will be $5{\cdot}5 \times 10^{-10}$ erg, which corresponds to a temperature of $5{\cdot}5 \times 10^{-10}/2 \times 10^{-16} = 2{\cdot}7 \times 10^{6}$ °C, a temperature which would generate quite a nice little nova!

The theory of thermo-nuclear reactions as applied to stellar conditions has been investigated by Atkinson (3), Gamow and Teller (4), Bethe (5), and others, and their results are equally valid for terrestrial conditions. The equation governing the rate of a thermo-nuclear reaction is according to their work;

$$p = \frac{4\varrho x_1 x_2 \Gamma}{3^{5/2} m_1 m_2 h} \cdot a R^2 \exp 4(2R/a)^{\frac{1}{2}} \cdot \gamma^2 \exp -\gamma$$

where p = number of processes per gram per sec.

ϱ = density of gas

x_1 and x_2 = the concentrations in grams per gram of mixture of the two reacting nuclear types,

m_1 and m_2 their masses,

R = combined radius

$a = h^2/m e^2 z_1 z_2$

m = reduced mass $m_1 m_2/m_1 + m_2$

$z_1 e$ and $z_2 e$ the nuclear charges

Γ/h = the probability of nuclear reaction in sec^{-1} after penetration

$$\gamma = 3\left[\frac{\pi^2 m e^4 z_1^2 z_2^2}{2h^2 k\theta}\right]^{\frac{1}{3}}$$

If we measure ϱ in g/cm^3, Γ in electron volts and θ in units of 10^6 °C, Bethe states that

$$\varrho = 5{\cdot}3 \times 10^{25} \varrho x_1 x_2 \Gamma \Phi (z_1 z_2) \gamma^2 \exp -\gamma$$

where $\gamma = 42{\cdot}7 (z_1 z_2)^{\frac{2}{3}} \left(\frac{A}{\theta}\right)^{\frac{1}{3}}$

$$\Phi = \frac{1}{A_1 A_2 (z_1 z_2 A)^3} \cdot \left(\frac{8R}{a}\right)^2 \exp 2 \left(\frac{8R}{a}\right)^{\frac{1}{2}}$$

$A = m/m_H$

m_H = mass of hydrogen nucleus

A_1 and A_2 are the chemical atomic weights of reacting types, and for the combined radius R he puts $1{\cdot}6 \times 10^{-13} (A_1 + A_2)^{\frac{1}{3}}$ cm.

In order to get a figure independent of the density and percentage composition of the reacting mixture, he calculates the value of P;

where $P = \frac{m_2}{\varrho x_1 x_2} \cdot p$

Since x_2/m_2 = number of nuclei of type 2 present per gram of mixture

$P \varrho x_1$ = probability per sec. that a given nucleus of type 2 undergoes reaction.

If we take a given mixture of two reacting types, i.e. fix ρ, x_1, and x_2, then γ is a function of θ only in expression for p, and $\gamma = b\theta^{-\frac{1}{3}}$ where b is a constant.

Calling ε_θ the energy production per g. per sec and Q the energy liberated per process, we see that ;

$\varepsilon_\theta = pQ = B\gamma^2 \, exp{-}\gamma$ where B is a constant.

This gives us the following simple relations,

(1) $\gamma = b\theta^{-\frac{1}{3}}$

(2) $\dfrac{\gamma_2}{\gamma_1} = \left(\dfrac{\theta_1}{\theta_2}\right)^3$

(3) $\dfrac{\varepsilon_2}{\varepsilon_1} = \left(\dfrac{\gamma_2}{\gamma_1}\right)^2 exp\ (\gamma_1 - \gamma_2)$

Hence if γ and ε are known for any temperature, we can easily find them for any other temperature, and also find the value of b.

Furthermore, since $\varepsilon = B\gamma^2 \, exp - \gamma$
ε is a maximum when $\gamma = 2$ i.e. when $\theta = b^3/8$

In applying these results to the sea, there are four reactions which must be considered. These are proton proton, proton deuteron, and deuteron deuteron reactions, the last of which may take two forms.

The nuclear equations are :

(1) ${}_1H^1 + {}_1H^1 = {}_1H^2 + e^+ + Q_1$
(2) ${}_1H^1 + {}_1D^2 = {}_2He^3 + Q_2$
(3) ${}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1 + Q_3$
(4) ${}_1D^2 + {}_1D^2 = = {}_1H^3 + {}_1H^1 + Q_4$

As far as the deuteron deuteron reactions are concerned, it will be sufficient to consider reaction 3 only, as it constitutes about 60% of the total reaction, and a factor of two in the results is hardly significant.

Bethe gives the estimated values of Q, and of γ and P at 20×10^6 °C. His figures are shown in Table I.

TABLE I.

Reaction	Q	γ_{20}	P_{20}
${}_1H^1 + {}_1H^1 = {}_1H^2 + e^+$	1·53 *mMU*	12·5	$8{\cdot}5 \times 10^{-21}$
${}_1H^1 + {}_1D^2 = {}_2He^3$	5·9 ,,	13·8	$1{\cdot}3 \times 10^{-2}$
${}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1$	3·5 ,,	15·7	10^3

1 *mMU* = 1 milli mass unit $= 0{\cdot}931 \times 10^6$ electron volts
$= 1{\cdot}49 \times 10^{-6}$ erg.

It is to be noted that the values of Γ used in obtaining these results are not of equal certainty. For reaction 1, Γ is calculated from Fermi's Theory of Positron Emission (6), for 2, Γ is apparently only estimated, whereas for 3, Γ is based on actual experimental determinations.

In using these results for terrestrial conditions, the value of ϱ to be used is obviously uncertain. It is clear, however, that it cannot exceed the density of the sea in the liquid phase, and accordingly in the following calculations I have taken $\varrho = 1\ g./c.c$, which will thus probably give a gross over-estimate of the rate of energy production. The concentration of heavy water in the sea is assumed to be 1/5000 that of ordinary water.

To show how the values of energy productions subsequently given have been obtained, I give one example, that of the proton proton collision.

Type of Reaction.

$$_1H^1 + {}_1H^1 = {}_1H^2 + e^+ + Q$$

$Q = 1{\cdot}53\ mMU = 2{\cdot}3 \times 10^{-6}$ erg

γ at $20 \times 10^6\ °C = \gamma_{20} = 12{\cdot}5$

P at $20 \times 10^6\ °C = P_{20} = 8{\cdot}5 \times 10^{-21}$

p at $20 \times 10^6\ °C = p_{20} = P_{20}\,\varrho\, x_1 x_2 / m_2$

$x_1 = x_2 = \frac{1}{9}$ nearly

$\varrho = 1\ g./c.c.$

$m_2 = 1{\cdot}56 \times 10^{-24}\ g.$

$$\text{Hence } p_{20} = P_{20}\,\frac{1 \times (0{\cdot}11)^2}{1.67 \times 10^{-24}} = P_{20} \times 7.2 \times 10^{21}$$

$$= 8.5 \times 10^{-21} \times 7{\cdot}2 \times 10^{21}$$

$$= 61.5$$

Hence the energy liberation per g, per sec

$$\varepsilon_{20} = p_{20} Q = 61{\cdot}5 \times 2.3 \times 10^{-6} \text{ erg}$$

$$= 1{\cdot}42 \times 10^{-4} \text{ erg per } g. \text{ per sec.}$$

Since we now have the values of γ and ε at $20 \times 10^6\ °C$ we can calculate ε at any temperature, and this has been done for a temperature of $1000 \times 10^6\ °C$ and also for the temperature of maximum generation of energy. The results are given in Table 2.

TABLE 2.

Reaction	ε_{20}	ε_{1000}	ε max	θ max
$_1H^1 + {}_1H^1 = {}_1H^2 + e^+$	$1{\cdot}42 \times 10^{-4}$	$8{\cdot}7 \times 10^{-2}$	$1{\cdot}33 \times 10^{-1}$	5270×10^6
$_1H^1 + {}_1D^2 = {}_2He^3$	$8{\cdot}5 \times 10^{10}$	$1{\cdot}36 \times 10^{14}$	$1{\cdot}90 \times 10^{14}$	6420×10^6
$_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1$	$7{\cdot}5 \times 10^{11}$	$6{\cdot}0 \times 10^{15}$	$1{\cdot}08 \times 10^{16}$	9670×10^6

These figures are to some extent surprising, showing that the deuteron deuteron reaction even in ordinary water is the most important one. This is due to the value of P_{20} which, however, is based on experimental determinations, and is thus the most certain value of P_{20} given for the three reactions concerned.

The best way, I think, of applying these results to the question of whether it is possible to initiate and maintain a thermo-nuclear reaction in the sea is to consider a rather artificial case. Supposing we take a sphere of water of density 1 *g./c.c.* and radius R in which thermo-nuclear energy is being generated at a rate of ε ergs per sec per g. Further let us assume that the sphere radiates as a black body. Then if the temperature is to remain constant; we have

$$\tfrac{4}{3}\,\pi\, R^3\varepsilon = 4\,\pi\, R^2\,\sigma\,\theta^4$$

where σ = Stefans Constant

Now $\varepsilon = B\gamma^2\, exp{-}\gamma$

$$\gamma = b\,\theta^{-\frac{1}{3}}$$

Hence $$R = \frac{3\,\sigma\,\theta^4}{Bb^2\,\theta^{-\frac{2}{3}}\, exp{-}b\,\theta^{-\frac{1}{3}}}$$

$$= C\,\theta^{\frac{14}{3}}\, exp\, b\,\theta^{-\frac{1}{3}}$$

where $C = 3\sigma/Bb^2$

It is easily shown by differentiating that R will be a minimum when $\gamma = 14$. For the deuteron deuteron reaction γ has this value when $\theta = 28 \times 10^6$ °C which gives the minimum value of R as $3{\cdot}1 \times 10^6$ km.

As this value is so large, there seems to be very little danger of starting a world wide explosion by dropping any possible atomic bomb into the sea. The experiment will probably be made before this paper is published.

It is of some interest to consider the case of pure heavy water. Table 3 gives the relevant figures.

TABLE 3.

Reaction	ε_{20}	ε_{1000}	ε max	θ max
${}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1$	$6{\cdot}8 \times 10^{19}$	$5{\cdot}45 \times 10^{23}$	$9{\cdot}8 \times 10^{23}$	9670×10^6

From these it can be shown that in this case the minimum radius of our hypothetical sphere would be only 3·6 km., so that even in an ocean of heavy water it would seem unlikely that a self-maintaining thermo-nuclear explosion could be started with an atomic bomb. It does seem probable, however, that if heavy water is incorporated in the bomb, some of the energy liberated in the explosion will be derived from a deuteron deuteron reaction, and that the neutrons emitted by the reaction would aid in ensuring the more complete fission of the active heavy element constituent.

In conclusion it may be pointed out that, although the method used in determining the critical radius of a self-maintaining thermo-nuclear explosion is physically very crude, it seems unlikely that any more complete investigation would lead to a large decrease in the critical radius, since in any actual explosion there will be a rapid decrease in density of the reacting components which would reduce the energy output. It is unlikely also that the assumption of black body radiation would be so far from the truth as to invalidate the conclusion that a self-maintaining thermo-nuclear reaction cannot be started in the sea by uranium fission.

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No. 8.

THE MECHANISM OF THE FORMATION OF ALLOPHANATES FROM CARBAMATES.

By

A. E. A. WERNER AND J. GRAY.

[Read December 18, 1945. Published separately May 6, 1946].

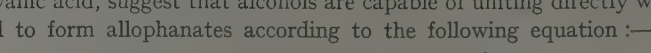
Since the discovery of ethyl allophanate by Liebig (1) numerous methods have been described for its preparation. These methods have been conveniently summarised by Bougault and Leboucq (2). Although the conversion of urethane into ethyl allophanate can be carried out by such reagents as sulphuryl chloride (3), phosgene (4), and phosphorus pentoxide (5), with varying yields, chief interest centres on the use of thionyl chloride. Schroeter and Lewinski (6) obtained ethyl allophanate by refluxing urethane with thionyl chloride in benzene solution, and later Warren and Wilson (7) repeated the reaction using a large excess of thionyl chloride in the absence of a solvent. In a recent communication one of us (A.E.A.W. 8) confirmed the work of Warren and Wilson but showed that it is not necessary to use a large excess of thionyl chloride, since urethane can be quantatively converted into ethyl allophanate by warming it at 60° C. for 1½ hours with just one molecular proportion of thionyl chloride. Very shortly after the publication of this communication Raiford and Freyermuth (9) confirmed the findings of Schroeter and Lewinski but reported that they were unable to obtain any ethyl allophanate according to the method of Wilson and Warren, obtaining instead only a small amount of cyanuric acid. In view of our results it is very difficult to understand the failure of Raiford and Freyermuth to obtain ethyl allophanate using Warren and Wilson's technique. Unfortunately they have omitted to give any experimental details ; the only explanation which suggests itself is the possibility that overheating may have occurred, since it has been shown (A.E.A.W. 8) that on heating ethyl allophanate is readily decomposed into cyanuric acid.

Since the reaction of Warren and Wilson as modified by us is of practical importance because it constitutes the best method for the preparation of ethyl allophanate, considerable interest also lies in the elucidation of the actual mechanism by which the reaction proceeds. Previous workers, including also Warren and Wilson (*loc. cit.*), without offering any explanation of the actual mechanism, contented themselves with the statement that the thionyl chloride acted as a de-

alcoholating agent, removing one molecule of ethyl alcohol from two molecules of urethane in accordance with the following equation :—

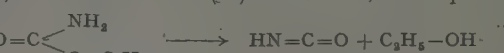
(1)

Davis and Blanchard (10), in a communication on the hypothetical substance dicyanic acid, suggest that alcohols are capable of uniting directly with this dicyanic acid to form allophanates according to the following equation :—



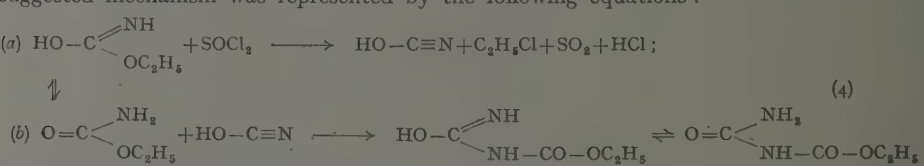
(2)

Although the experimental conditions would not be favourable to the formation of a substance apparently so unstable as dicyanic acid, the possibility might still remain that the thionyl chloride functions by accelerating the decomposition of the urethane molecule at a lower temperature in a manner similar to its thermal decomposition which, as E. A. Werner (11) has shown, takes place slowly thus :—



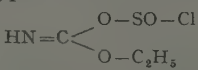
(3)

The cyanic acid thus formed might, according to the hypothesis of Davis and Blanchard, dimerise and combine with alcohol to give ethyl allophanate. Since the existence of the substance dicyanic acid must be regarded as very doubtful,[1] an alternative mechanism was put forward in our original communication in which it was tentatively suggested that the formation of ethyl allophanate proceeds in two stages. In the first stage it was assumed that cyanic acid was liberated in an "active" form from one molecule of urethane, followed in the second stage by the addition of this "active" cyanic acid molecule to unchanged urethane. This suggested mechanism was represented by the following equations :—



(4)

which proceed simultaneously after the initial decomposition of the urethane has started. Raiford and Freyermuth (*loc. cit.*) incidentally suggested a similar mechanism and showed that the thionyl chloride initially reacted with urethane to produce an intermediate complex of the type



which then decomposed into sulphur dioxide, ethyl chloride, and cyanic acid, which was assumed to combine with unchanged urethane to give ethyl allophanate.

It is well known that cyanic acid readily combines with alcohols in neutral solution forming carbamates, or with amines forming substituted ureas; there is, however, no direct experimental evidence to show that cyanic acid is normally

[1] We have repeated the experiments described by Davis and Blanchard to show the existence in solution of dicyanic acid, but have been unable to confirm their results. This work will be the subject of a forthcoming communication.

capable of reacting with the carbonamide group, as postulated in the second stage of the mechanism suggested in our original communication. It was for this reason that an ' active ' form of cyanic acid was originally suggested as being necessary. The present communication is therefore concerned with the presentation of additional experimental evidence to support the suggested mechanism, and to show that under suitably chosen conditions cyanic acid is liberated in an activated form, which readily reacts with the carbonamide group.

A series of experiments was carried out in which cyanic acid was generated by different methods and allowed to react directly with urethane under different experimental conditions, so chosen that they would permit of an unambiguous interpretation. Under certain conditions it was found that no ethyl allophanate was formed, but if the correct experimental conditions were chosen direct addition of cyanic acid to the carbonamide group of the urethane molecule occurred, leading to the formation of ethyl allophanate in good yield. The actual techniques employed may be conveniently discussed under the following headings :—

(i) *Reaction between cyanic acid gas and urethane in neutral media.* Hofmann (12) claimed that ethyl allophanate was formed by passing cyanic acid vapour directly into molten urethane, but he himself did not give any experimental evidence in support of his statement. Davis and Blanchard (*loc. cit.*) state that Béhal (13) failed to confirm Hofmann's result ; but this is incorrect, since Béhal does not mention having repeated Hofmann's work, and in point of fact a survey of the chemical literature has shown that Hofmann's claim has never been experimentally tested. According to our experimental results, however, no trace of ethyl allophanate was produced when cyanic acid gas was passed into molten urethane. In each case the sole products of the reaction were cyanuric acid and unchanged urethane. A similar result was obtained when an ethereal solution of cyanic acid was added to an ethereal solution of urethane at room temperature ; after a few days the only insoluble product that settled out was cyanuric acid. From these results it may be reasonably concluded that in neutral media cyanic acid does *not* react with urethane.

(ii) *Cyanic acid generated in glacial acetic acid solution.* Bailey and his co-workers (14) have shown that, in the case of a number of weak nitrogen bases, carbamide derivatives are readily formed in glacial acetic solution by stirring in finely ground potassium cyanate, and they have demonstrated the usefulness of this modified form of the Wohler synthesis in many cases. This technique was applied by us, but the expected addition reaction between urethane and cyanic acid did not take place ; only ill-defined polymerisation products were obtained, and no trace of ethyl allophanate could be detected.

(iii). *Cyanic acid generated by the thermal decomposition of urea.* Since ethyl allophanate is produced in large yield by the action of thionyl chloride on an equimolecular mixture of urea and urethane, as shown in our original communication, an experiment was now carried out in which an equimolecular mixture of urea and urethane was heated at a temperature of 145°–170° C. for a few hours. The result of this experiment was, however, in direct contrast to that in which thionyl chloride was used. No trace of ethyl allophanate could be detected among the reaction products, which were found to consist of unchanged urethane, biuret, and cyanuric acid. This result is in agreement with the work of Davis and Blanchard (*loc. cit.*),

who failed to obtain *n*-butyl allophanate when they heated *n*-butyl carbamate with urea or nitrourea. Their interpretation of this result as proving that allophanates are not formed by addition of cyanic acid to carbamates, but are formed directly from dicyanic acid and an alcohol is however incorrect. The true explanation of the non-formation of ethyl allophanate is given later in the text.

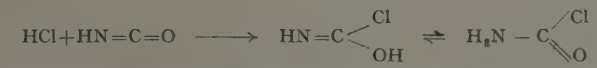
In contrast to the above experimental results it was found that ethyl allophanate was readily produced when cyanic acid was generated in the presence of hydrochloric acid. In the first series of experiments *dry* hydrochloric acid gas was passed into a suspension of potassium cyanate in molten urethane, and ethyl allophanate was produced in good yields. The yield decreased with rise in temperature, and at 140° C. amounted to only 7% of the theoretical. This decrease in the yield of ethyl allophanate with increase in temperature is due to the fact that at the higher temperature the urethane is largely decomposed by the hydrochloric acid, as shown by E. A. Werner (*loc. cit.*), according to the following equation :—

$$O=C\begin{matrix}NH_2\\O-C_2H_5\end{matrix} + 2HCl \longrightarrow CO_2 + C_2H_5Cl + NH_4Cl \qquad (5)$$

Our experimental results confirm this conclusion. At 50° C. there is no appreciable decomposition when hydrochloric acid gas is passed into molten urethane ; at 80° C. decomposition is appreciable, and from the weight of ammonium chloride formed it is possible to calculate the amount of urethane decomposed in this manner, and finally at 140° C. decomposition is so rapid that a copious precipitate of ammonium chloride forms immediately. Since no cyanuric acid is produced in these experiments it is clear that thermal decomposition of urethane according to equation (3) does not occur. This disposes of any possibility that the ethyl allophanate could be formed by the mechanism favoured by Davis and Blanchard (*loc. cit.*). Furthermore, if the formation of ethyl allophanate were connected with the decomposition of the urethane molecule, the yield of ethyl allophanate should be zero at 50° C. and a maximum at 140° C. In point of fact the experimental findings are quite the reverse, thus indicating that the ethyl allophanate is actually formed by the direct union of cyanic acid with the carbonamide group of the urethane molecule.

This conclusion received further experimental support from a final series of experiments in which cyanates of potassium, sodium, or silver were added to a solution of urethane in *anhydrous* ether which had been saturated with *dry* hydrochloric acid gas. After the reaction mixtures had stood at room temperature for a few hours a crystalline precipitate of ethyl allophanate was collected, the yield amounting to about 70% of the theoretical. The reaction also proceeded satisfactorily using anhydrous benzene or anhydrous dioxane as solvents. This technique is so simple and gives such satisfactory results that it was extended to the synthesis of a whole series of allophanates from the corresponding carbamates as described in the experimental part.

This final series of experiments affords conclusive evidence that it is possible to bring about the direct addition of cyanic acid to the carbonamide group $-CONH_2$, if the cyanic acid is liberated in the presence of hydrochloric acid. An active complex, namely nascent carbamyl chloride, is formed '*in situ*' according to the following equation :—



The nascent carbamyl chloride behaves like an acid chloride and reacts immediately with urethane forming ethyl allophanate, and regenerating hydrochloric acid, thus :—

The above explanation of our experimental results receives further support when considered in the light of the work of Gattermann, who demonstrated the chemical reactivity of carbamyl chloride (prepared by the action of phosgene on ammonium chloride at 400° C.). In the first communication (15) he showed, *inter alia*, that if ethyl alcohol is slowly added to liquid carbamyl chloride, ethyl allophanate is readily formed. Although he represented this reaction by the equation :—

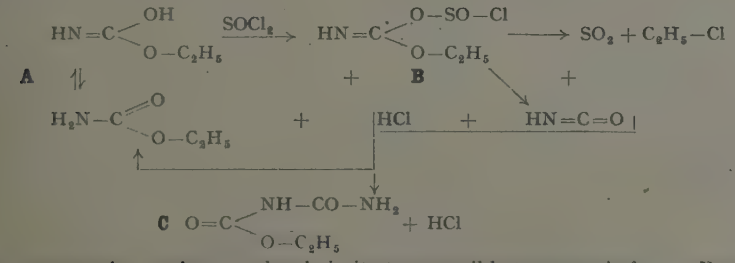
$$2Cl{-}CO{-}NH_2 + C_2H_5OH = 2HCl + H_2N{-}CO{-}NH{-}CO{-}OC_2H_5$$

it undoubtedly involves the initial formation of urethane with which the excess carbamyl chloride combines to form the ethyl allophanate. In a subsequent communication (16) Gattermann showed that aromatic acid amides can be synthesised by passing a current of dry hydrochloric acid and cyanic acid gases simultaneously into an aromatic hydrocarbon in which anhydrous aluminium chloride is suspended. Thus, for example, benzamide is formed from benzene according to the following equation :—

$$C_6H_6 + HN{=}C{=}O + [HCl + AlCl_3] = C_6H_5CO{-}NH_2 + [HCl + AlCl_3]$$

These illustrations of the chemical reactivity of carbamyl chloride would seem to be of sufficient interest to warrant further investigation and the action of this reagent on other carbonamide derivatives will be the subject of a future communication.

When the additional experimental evidence presented in this communication concerning the addition of cyanic acid to the carbonamide group is taken in conjunction with the evidence of Raiford and Freyermuth (*loc. cit.*) concerning the nature of the initial intermediate complex formed, it enables one to form a complete picture of the mechanism involved in the formation of ethyl allophanate by treating urethane with thionyl chloride. The complete mechanism may be schematically represented as follows :—



A represents the urethane molecule in its two possible tautomeric forms, B represents the intermediate complex, which decomposes into sulphur dioxide, ethyl chloride, and cyanic acid ; the latter reacts with unchanged urethane in the presence of hydrochloric acid to give ethyl allophanate C.

Experimental.

Reaction between cyanic acid gas and urethane. (i). *In molten state.* Cyanuric acid (1·5 g.), previously dehydrated by drying at 110° C., was heated to dull red heat in a hard glass tube in a tubular electric furnace. The cyanic acid vapour was passed into urethane (5 g.) contained in a U-tube maintained at a temperature of 55° C. An opalescence developed in the molten urethane, and after extraction with ether an insoluble residue remained which was identified as cyanuric acid by the usual tests. A similar result was obtained when the reaction was repeated at 80° C. and 140° C.

(ii). *In ethereal solution.* To four tubes containing an ethereal colution of cyanic acid (0·6 g.) urethane (1 g., 2 g., 3g., and 5 g.) was added. After standing a few days at room temperature a white precipitate settled out which was identified as cyanuric acid.

Reaction between cyanic acid and urethane in glacial acetic acid. To a solution of urethane (8·9 g.) in glacial acetic acid (30 c.c.) potassium cyanate (8·1 g.) was slowly added. The resultant semi-solid mass was warmed on the water-bath for one hour. Subsequent treatment with ether to remove unchanged urethane caused the precipitation of a crystalline substance, M.P. 110° C., identified as a molecular compound of acetic acid and potassium acetate (cf. Beilstein. vol. II, p. 108). After extraction with distilled water a residue of ill-defined polymerisation products of cyanic acid remained from which boiling benzene failed to extract any ethyl allophanate.

Action of heat on an equimolecular mixture of urea and urethane. An intimate mixture of urea (9 g.) and urethane (14 g.) was heated in an oil-bath. A homogeneous melt formed at 95° C.; reaction started at 160°–170° C. with copious evolution of ammonia, ethyl alcohol distilled over and a white solid separated. The heating was continued for three hours. Ethereal extraction removed unchanged urethane (0·5 g.) leaving a solid residue (13·5 g.), which was treated with a volume of caustic soda solution just sufficient to cause complete solution. Passage of carbon dioxide through this solution precipitated biuret in needle-like crystals (5 g.). Further acidification with dilute acid precipitated cyanuric acid (8 g.). No ethyl allophanate could be detected.

Reaction between cyanic acid and urethane in presence of hydrochloric acid (i). *In molten state.* Dry finely powdered sodium cyanate (10·6 g.) was suspended in molten urethane (15 g.) maintained at 80° C., and dry hydrochloric acid gas was passed in for 30 minutes. When cool the contents of the flask were extracted with ether to remove unchanged urethane (10·9 g.). The residue was treated with cold water (75 c.c.) and filtered. The amount of undecomposed sodium cyanate in the filtrate was determined and found to be 5·28 g. The remaining insoluble residue was dissolved in hot water, and titrated while still hot with N sodium hydroxide, converting any cyanuric acid present into soluble sodium hydrogen cyanurate. On cooling a white microcrystalline precipitate settled out. Weight, 3·9 g. M.P. 193° C., not depressed by mixture with an authentic specimen of ethyl allophanate. Upon acidification cyanuric acid (1·6 g.) was obtained. From the above data it follows that the amount of cyanic acid actually liberated was 3·55 g., of which 1·6 g.

polymerised to cyanuric acid, leaving 1·95 g. available for union with urethane to give ethyl allophanate (theoretical yield=5·99 g.). Hence actual yield of ethyl allophanate is 65% of the theoretical. The formation of this amount of ethyl allophanate would require 2·63 g. of urethane. Hence weight of urethane directly decomposed by the action of the hydrochloric acid was 1·47 g.

(ii). *In ether or dioxane solution.* (a) Urethane (8·9 g.) was dissolved in anhydrous ether (50 c.c.) saturated with dry hydrochloric acid gas (HCl=ca. 3N) contained in a conical flask closed with a calcium chloride tube. Dry finely powdered sodium cyanate (6.5 g.) was added slowly in small amounts. After the mixture had stood overnight, the supernatent ethereal layer was decanted and the solid residue drained on the filter-pump. After extraction with cold water (50 c.c.) to remove sodium chloride an insoluble residue remained which melted at 193° C., and showed no depression on mixing with an authentic specimen of ethyl allophanate Weight, 7·9 g., corresponding to 67% of the theoretical.

(b) To a solution of urethane (4·5 g.) in anhydrous dioxane saturated with hydrochloric acid (HCl=5N) silver cyanate (7·5 g.) was added slowly. After standing overnight the dioxane layer was decanted, and the silver chloride residue extracted twice with hot water. The hot filtrate deposited microcrystalline ethyl allophanate, M.P. 193° C. Weight, 3·7 g., corresponding to 56% of the theoretical.

Decomposition of urethane by hydrochloric acid. Dry hydrochloric acid was passed for 30 minutes into molten urethane (5 g.) contained in a wide-necked flask maintained at the following temperatures :—(i) *At* 50° C. : No reaction occurred, the contents of the flask were completely soluble in ether. (ii) *At* 80° C. : A slight reaction occurred, and the residue left after extraction with ether consisted of pure ammonium chloride. Weight, 0·82 g. Tests for the presence of ammonium cyanate or cyanuric acid were negative. (iii) *At* 140° C. : A copious precipitate of ammonium chloride formed immediately. The weight, after removal of unchanged urethane, was 1·9 g., corresponding to 63% of the theoretical. Even at this elevated temperature no trace of ammonium cyanate or cyanuric acid could be detected.

Preparation of allophanates from the corresponding carbamates. In each case the preparation was carried out by adding an equimolecular proportion of sodium cyanate to an equimolecular proportion of the carbamate dissolved in ether or dioxane saturated with dry hydrochloric acid. Unchanged carbamate was removed by extracting the solid residue with cold alcohol, and the allophanate was purified by recrystallisation from hot water or alcohol. In this manner the following allophanates were prepared in excellent yields, and identified by their M.P.s which agree with those given in the literature : Methyl M.P. 210° C. ; *n*-Propyl M.P. 175° C. ; *iso*-Propyl M.P. 179° C. ; *n*-Butyl M.P. 149° C. and *iso*-Amyl M.P. 160° C. The successful outcome of these preparations necessitates the use of anhydrous reagents and the complete exclusion of moisture, in order to prevent hydrolysis of the carbamyl chloride to ammonium chloride and carbon dioxide.

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No. 9.

DOPPLERITE IN CO. LIMERICK.

M. Dee and G. F. Mitchell.

[Read November 27, 1945. Published separately May 7, 1946].

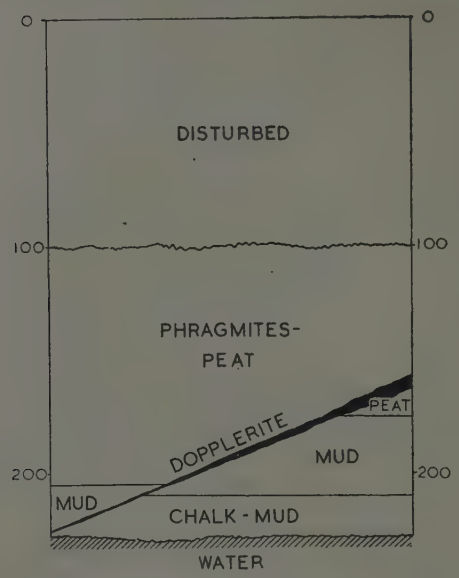
While engaged in superintending the production of turf in Co. Limerick in 1944 one of the authors (M.D.) noticed that the turf being used in a forge at Lisnagry contained a black pitchy-looking substance that burnt very vigorously. Enquiries revealed that the turf came from a cutting in a nearby bog in Mountshannon Townland (6″ O.S. Map Limerick Sheet 6 ; 22·5 cm. from top, 33·5 cm. from left) where fresh dopplerite could be seen in a vein-like mass intersecting the peat face. The authors made a detailed examination of the site in August, 1945. As previous records of the occurrence of this mineral in Ireland are few in number the discovery seemed worthy of record. In 1903 Moss (1) recorded dopplerite from a bog in Co. Antrim, and there are two records (2 & 3) prior to 1850 (when the mineral was first described and named) which certainly refer to dopplerite.

In the area north-east of Limerick city there are large expanses of raised-bog, which have been extensively cut away in the vicinity of Lisnagry. That the cut-away bog in Mountshannon Townland was originally a raised-bog was shown by a surviving block of isolated peat, which had the typical stratigraphy :—

Fresh *Sphagnum*-peat.
Highly-humified *Sphagnum*-peat.
Phragmites-peat rich in wood (Marsh-peat).
Mud (laid down in open water).
Chalk-mud (laid down in open water).
Sandy clay (of glacial origin).

In the immediate vicinity of the cutting showing the dopplerite all the fresh *Sphagnum*-peat and most of the highly-humified *Sphagnum*-peat had been cut away. The existing cuttings (in *Phragmites*-peat) go below the local water-table and fill with water as soon as cutting ceases. When as much water as possible had been pumped out the section illustrated was seen in a corner of the cutting.

The upper metre of peat was disturbed material from former cuttings, and underneath this was *Phragmites*-peat. It was clear that the upper part of the



Section in turf-cutting, showing dopplerite lying above a fault in the turf. Depths in centimetres.

Phragmites-peat in the section had been faulted downwards and towards the north in relation to the lower part of the section, the vertical movement being about 30 cm. Due to the disturbance of the surface it was not possible to trace the fault beyond the limits of the cutting which was 6 m. long. There were deep cuttings (now abandoned) to the north of the cutting, and the fault had presumably developed after these cuttings removed support from the north side of the remaining peat. The effect of the fault was to bring the *Phragmites*-peat which was stratigraphically above the mud down into lateral contact with the mud.

The dopplerite had developed in the plane along which movement had taken place and so occurred in a vein-like mass. It had developed most freely (to a thickness of 6 cm.) where peat remained in contact with peat across the fault, it was less thick where peat had been faulted against mud, and only appeared as a thin streak where mud remained in contact with mud across the fault. In some places patches of apparently unaltered peat were completely surrounded by dopplerite : in others, where stems of *Phragmites* still surrounded by peat abutted against the main mass of the dopplerite, the cavity in the stem for some little distance away from the contact was found to be filled with dopplerite.

These observations suggested that the dopplerite was of recent formation, being subsequent to the earlier cuttings and the development of the fault. Its presence where peat abutted against the fault and its absence where mud only abutted against the fault suggested that the dopplerite had been formed from the constituents of the peat. The plane of the fault would allow of freer movement of

gases and liquids than would be normally possible in peat or mud, and such a circulation may have assisted the formation of the dopplerite. In a laboratory experiment the introduction of some uncontaminated dopplerite into an agar plate was followed by a growth of bacterial colonies and a marked liquifaction of the agar in the vicinity of the colonies. It is possible that in the alteration of peat to dopplerite bacterial action may play a part.

Schreiber (4) in his book *Moorkunde** discusses the occurrence of dopplerite in bogs on the continent. He states that it occurs most frequently in gaps, veins, and fissures, and in the vicinity of pieces of wood and plant stems, especially filling up cavities in the latter. He explains the origin of the fissures as usually caused by a shrinking of the bog due to a dry climatic phase, the fissure being later filled with peat when the bog growth was resumed. He makes no reference to the formation of fissures by faulting in the peat. He considers that the dopplerite is often in a secondary position, as it is sometimes found, not only in the peat itself, but also in the sub-soil underneath the bog. One of the early Irish records (3) describes 'a substance externally resembling bitu. n' of . jetty black colour—soft and spongy when first removed from the gravel, with a conchoidal fracture' (almost certainly dopplerite) in the gravel below a bog in Co. Kildare. Schreiber pictures the cell-walls of the plant-tissues decaying into dopplerite, and the mineral being carried by water into suitable resting-places, though he does not deny the possibility that it may sometimes occur *in situ*. Abroad, as at Lisnagry, it occurs most frequently in *Phragmites* peat but has also been found in peat built up of moss remains. The position of the dopplerite at Lisnagry makes it almost certain that it must have been derived from the *Phragmites* peat and not from some other peat from whence it had migrated into its present position. The fault (along the plane of which it lies) could not have developed until extensive cutting had removed upper layers of peat leaving only *Phragmites* peat *in situ*. Also the nuggets of apparently unaltered peat entirely surrounded by dopplerite suggest that a progressive alteration was moving out from the plane of the fault. Dopplerite was scarce wherever mud was in contact with mud. This mud, formed chiefly of the *excreta* of the *plankton* that lived in the open water of the lake that preceded the marsh, is poor in cellulose, and this may explain the paucity of dopplerite in its vicinity, The calcium carbonate in the underlying chalk-mud was probably deposited by *Chara*, but this chalk-mud was also poor in cellulose.

Two of the earlier Irish records (1 & 3) place the dopplerite at or below the bottom of bogs that had been disturbed by human activities. This seems to be the typical location on the continent also, and it is possible that dopplerite may form where an alteration in the natural conditions allows gases and liquids to percolate more freely. This circulation, perhaps assisted by bacteria, brings about the transformation of plant debris to amorphous jelly. Dopplerite is therefore of recent origin, and cannot be regarded as a substance of high antiquity, or as an intermediate stage in the transformation of peat to coal.

*The authors are indebted to Dr. J. Lennon of the Industrial Research Council for drawing their attention to this reference.

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No. 10.

OBSERVATIONS ON THE PASMO DISEASE OF FLAX AND ON THE CAUSAL FUNGUS *SPHAERELLA LINORUM* WOLLENWEBER.

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(Plates 2, 3 and 4).

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Introduction.

Pásmo is a Spanish word meaning spasm, and is the common name given to a disease of flax which was first described by Spegazzini (12) in the Argentine in 1911, the causal fungus being then named *Phlyctaena linicola*. The disease was afterwards recorded and described by Brentzel (2) in the U.S.A. The first record of the disease in the old world is apparently that by Rost (11) who in 1936 reported it as causing heavy damage to flax in Yugoslavia. He named the fungus *Septoria linicola*. Wollenweber (15) in 1938 found and described the perithecial stage of the parasite responsible for pasmo, and as a result changed the name of the fungus to *Sphaerella linorum*. During the last ten years the disease has spread extensively throughout many of the flax-growing regions of the world, being reported from Germany (14), Denmark (1), Hungary (5), Russia (8). Kenya (9), and New Zealand (4), Colhoun and Muskett (3) worked with pasmo-infected material received from Kenya, but beyond identifying the organism and proving its pathogenicity under greenhouse conditions no detailed investigations of the disease was made under the circumstances.

The first occurrence of Pasmo in Ireland is that reported by Lafferty and McKay (7) in 1944. They found a fungus growing on wild flax, *Linum angustifolium*, on a farm in the South of Ireland. Morphologically this fungus corresponded with published descriptions of *S. linorum*, and cross inoculations by means of spore suspensions showed that the fungus was capable of causing pasmo symptoms on cultivated flax, thus leaving little doubt as to the identity of the organism.

Following this report a survey of flax diseases was undertaken in the twenty-six counties in 1945—a report of which will appear elsewhere—and at the same time a study of pasmo on flax was carried out at the Albert Agricultural College, Glasnevin. The latter study together with an account of the known distribution of the disease in Ireland constitute the subject of the present paper.

Investigation of the Disease at Glasnevin.

Symptoms on Seedlings.—In October, 1944, pycnospores derived from a single spore culture of *S. linorum* originally isolated from *L. angustifolium* were suspended in sterile water and sprayed by means of an atomiser on to flax seedlings of the variety Liral Monarch growing in pots of sterile soil in a glasshouse. The pots were covered with belljars for ten days after inoculation so that a very moist atmosphere was maintained around the seedlings. Similar pots of seedlings sprayed with sterile water were kept under the same conditions and acted as controls. The first symptoms appeared on the inoculated seedlings at the end of eleven days as minute circular pale spots on the cotyledons. These lesions enlarged rapidly and developed a water-soaked appearance, later becoming grey or silvery in colour and somewhat irregular in shape (Fig. 1, Plate 2). Sixteen days after inoculation whole cotyledons were withered and hanging down on the plants, and after a further five days abundant black pycnidia developed upon them (Fig. 2, Plate 2). These pycnidia gave rise to the characteristic pycnospores of *S. linorum*. There was no evidence that the fungus had spread upwards along the plants and five months after inoculation there was no trace of the disease beyond diseased areas on the stems below the point of attachment of the cotyledons. This, however, is not surprising, as under the conditions existing in the glasshouse there was no means by which the spores could have been disseminated. Furthermore, it was later found, and the fact has been commented upon by other workers on this subject, that flax plants between the cotyledonary and flowering stages are very resistant to infection by *S. linorum*.

Confirmation of the temporary resistance of flax to infection by this parasite was obtained from observation on crops which developed from naturally infected seedlings. The first flax seedlings with pasmo disease came to hand early in May, 1945. The attack was quite severe, resembling that of Seedling Blight caused by *Colletotrichum linicolum*. An examination of the crop from which these seedlings came was carried out in the month of June. At this time the presence of the disease could not be verified by field inspection, and it was only when suspicious-looking plants were brought to the laboratory and examined microscopically that the presence of the disease was confirmed. Moreover in the case of a crop of oil flax of the variety Redwing the disease did not attract any attention until September, when the crop was almost mature.

Symptoms on Maturing Plants.

On flax plants which have reached the stage of boll formation, the most striking symptom is a pronounced blight (Fig. 3, Plate 2). This is brought about by the destruction of the leaves on which numerous lesions develop. These diseased areas

are at first somewhat circular in shape, of a brownish grey colour, sometimes with a lighter centre, and are surrounded by a narrow band of chlorotic tissue. Frequently the lesions enlarge in size so that they coalesce, and the whole leaf is rapidly killed. In the final stage the leaf lesions become bleached, especially towards the centre, and numerous black pycnidia develop upon them (Fig. 4, Plate 2). These pycnidia are easily seen against the light-coloured background of the diseased tissue. On the stems infection occurs at any point, and the diseased areas are elliptical in shape, rarely completely surrounding the entire stem, and vary in length from one quarter to one inch (Fig. 5, Plate 2). In colour they are at first dark red or purple, later becoming brownish and sometimes tending to bleach out to a light colour. Pycnidia of the fungus develop also on the stem lesions, and under very moist conditions tendrils of pycnospores exude from them. These spore tendrils are horn-like or coiled, and when they are numerous they give a hairy appearance to the diseased areas (Fig. 8, Plate 3). Similar symptoms occur on the flowering branches. The sepals are extremely susceptible to infection, and when diseased they at first turn brown. Later the colour becomes light or silvery, and pycnidia develop profusely upon them (Fig 6, Plate 3). This bleaching of the sepals with the pycnidial development is a rather striking symptom of the disease. The sepals appear to become entirely permeated by the parasite, and from them the pedicel at its junction with the boll is invaded. This invasion of the pedicel is significant, as will be seen later.

In the final stages of the disease, the leaves fall off, and the bare stems present a mottled appearance compounded of the light green of the healthy portions of the stems and the darker colour of the lesions. In the case of plants which became attacked about flowering time the bolls remain small in size, and the seed from such bolls is somewhat shrivelled, light, and discoloured.

Under favourable conditions the disease spreads from plant to plant with great rapidity. Such favourable conditions include high atmospheric humidity or heavy rainfall occurring at the susceptible stage in the growth of the host plant. Spread of the parasite under these conditions seems to be brought about mainly by wind-borne spores, although rain splashes or insects would no doubt play a part in transferring spores from diseased to neighbouring healthy plants.

The Fungus.

The source of the fungus in the present investigations was a wild flax plant from which Lafferty and McKay had recorded the disease. A pure culture of the parasite originating from a single spore was obtained by the dilution method, and this culture or sub-cultures from it were used throughout the work. The fungus grows readily on potato glucose agar, the medium on which the growth of the fungus is described. The rate of growth is slow, colonies reaching a diameter of 1.50 cm, when incubated for 10 days at 21°C. Such colonies are roughly circular in shape with an entire or very slightly lobed or wavy margin. The centre of each colony is almost black, the texture of the medium is somewhat tough, and the surface is overgrown with whitish aerial mycelium. Cream to orange coloured beads of pycnospores occur towards the centre of each colony (Fig. 14, Plate 4), and these

arise from pycnidia which are submerged in the medium. When colonies are crowded upon a plate they remain small, usually not exceeding 0·5 cm. in diameter. A few pycnidia are produced in such colonies.

The pycnidia of *S. linorum* are almost black in colour with a definite wall, and occur submerged in the culture medium or in the host tissue. They open through a round ostiole which when complete is 30 – 45 μ in diameter. The pycnidia vary in diameter from 60 μ up to 135 μ and average 95 μ.

The pycnospores (Fig. 11, Plate 3) are curved, straight, or slightly bent, with rounded ends. They are hyaline and are generally 3 – septate although individuals have been noted with up to five septa. The size of the pycnospores varies, the average being 26 μ × 2·8 μ and the extremes 21 μ × 2·5 μ and 33 μ × 3·3μ. Pycnospores from a flax stem collected in Co. Cork averaged 31 μ × 4·1 μ the extreme limits being 30 μ × 4 μ and 35 μ × 4·5 μ.

Pycnospores germinate readily in water or on slides in a saturated atmosphere, and after 24 hours at room temperature produce germ tubes 20 μ to 40 μ long. These in the majority of cases arise from the terminal cells, but they may arise from any cell. A rather striking feature of the germinated pycnospores is their power to conjugate or anastomose (Fig 12, Plate 4), but the significance of this is not understood. Another type of germination was observed where the pycnospore budded off an elliptical secondary spore about 10 μ in length, which having become detached germinated in turn by the production of a thin germ tube from either or both ends.

Brentzel (2) in the U.S.A. has described and figured from artificial cultures of *S. linorum* bodies resembling chlamydospores, and found that they were capable of germinating under suitable conditions. The present authors have found bodies of a similar nature occurring amongst pycnospores not alone from old cultures, but also from diseased stems, seeds and boll tissue. These chlamydospores are generally rounded in shape, wider than the normal width of the cells of the pycnospore which give rise to them, and with slightly thicker walls than the latter (Fig. 13, Plate 4). Frequently the contents of an adjoining cell appear to be absorbed in the formation of a chlamydospore, but pycnospores have been observed where each of the four cells developed into a chlamydospore. The chlamydospores in turn become divided by a cross wall, so that each forms two separate spores. They germinate in the same manner as pycnospores, but the germ tubes arising from them are generally stouter than those from the latter. Chlamydospores are said to be resistant to conditions which may be adverse to the fungus, and may thus be a means of ensuring the persistence of the parasite during the winter or unfavourable periods—such as prolonged drought.

Dissemination of the Disease by the Seed.

Most workers on the subject have reached the conclusion that pasmo disease of flax is seed borne. Thus Brentzel (2) found pycnospores of *S. linorum* in washings of seed from an infected crop, and as a result he concluded that the parasite is carried as a contamination on the surface of the seed. Wollenweber (15), advancing as evidence the observation that in pasmo pycnidia occur on the seed capsules, concluded that the fungus is carried on the seed. Nattrass (9), on the

other hand, failed to find *S. linorum* in centrifuged washings of seed from a diseased crop, but nevertheless considers it safest to assume that the fungus is seed-borne, and believes that small particles of infected material may carry pycnidia and dried spore masses. The present authors have found that, when seed was separated from crushed infected bolls and shaken up in a small quantity of water which was later centrifuged, the sediment contained numerous pycnospores of *S. linorum* It has never, so far as the authors are aware, been proved experimentally that such contaminated seed could give rise to the pasmo disease, and it is rather significant that the majority of workers on this disease refer to the great difficulty of controlling it by seed treatment. Amongst these may be mentioned Rost (11), Stapel (13), and Newhook (10). Stapel points out that the fungicidal action of the plant-protectives rose as the quantities were increased, but a very adverse influence was exerted on germination and growth of the seed, and such treatments are not practicable on a large scale. Newhook in tests carried out in New Zealand with hot water and four dusts found that only the former gave any measure of control, immersion of the seed for ten minutes being necessary at a temperature of 127°F.

While pasmo may be spread by detritus amongst the seed or by spores adhering to the surface of the seed these are not the only methods of seed dissemination, nor are they the most important ones, as will now be shown.

Seed Infection.

A careful examination of numerous bolls from infected flax plants was made, but on none was there any indication that the pericarp or wall of the boll was invaded by the parasite. This fact led to the conclusion that if seed became infected it could do so only by invasion from the placenta through the funicle. As already stated, in the case of flax plants affected by pasmo the sepals become permeated by the fungus. It was later observed that in almost all cases where this occurred the parasite made its way from the diseased sepals to the pedicel at its junction with the boll. When the bolls borne on such diseased pedicels were broken open many of them had pycnidia developed in profusion along the placenta and on the dissepiment (Figs. 15 & 17, Plate 4). Furthermore, many of these pycnidia had produced extremely long spore tendrils (Fig. 7, Plate 3). These tendrils at the time of examination had become hardened and firm, and many of them could be seen with the naked eye. When the seed from such infected bolls was examined with the aid of a binocular dissecting microscope incipient pycnidia could be seen on the testa in the region of the hilum. These pycnidia developed rapidly (Fig 9, Plate 3) when the seed bearing them was incubated in a moist chamber, and at the end of twenty-four hours many of them were exuding pycnospores (Fig. 10, Plate 3) which on examination proved to belong to *S. linorum*. It is noteworthy that a similar type of seed-infection was found to occur in the case of *L. angustifolium*, and many seeds bearing pycnidia of *S. linorum* were obtained from diseased plants of this species.

The path of the fungus, therefore, appears to be from the sepals to the pedicel, thence to the placenta, and so to the seed through the funicle. Further confirmation of the conclusion that flax seed becomes infected with *S. linorum* in this way was

forthcoming when serial sections were made of diseased seeds. It was proved in all cases where mature seed was used that the pycnidia were confined to the seed coat in the immediate region of the hilum. A very few seeds were observed on which pycnidia developed at other points, but such seeds proved to be immature with imperfectly developed coats, and were incapable of germination. Examination of sections of infected seeds showed that the pycnidium arises as a stroma between the round cells and the fibrous layers of the seed coat, and as it enlarges the former are pushed outwards and lie along the wall of the pycnidium (Fig. 16, Plate 4). The epidermal layer which is permeated by the mycelium is finally penetrated by the enlarging pycnidium, the ostiole of which pushes through the cuticle. There is no conclusive evidence that the parasite penetrated the fibrous layers of the seed coat, and there was no trace of injury to the endosperm or cotyledons. This would account for the fact that although an infected seed might bear numerous pycnidia in its testa it nevertheless germinates in a normal manner.

Seed which was carefully removed by hand from flax bolls bearing pycnidia of *S. linorum* on sepals and pedicels was placed in a germinator at room temperature. After three days 80 per cent. of the seed had germinated, and of these 20 per cent. had developed pycnidia of *S. linorum* on their coats. Fourteen days later half of those which had developed fructifications showed water-soaked lesions on their cotyledons, and these eventually bore pycnidia of the fungus. In another experiment 100 seeds from the same source as above were sown in a pot of sterilised soil in the glasshouse and germinated to the extent of 92 per cent. The pot was covered with a bell jar to exclude possible extraneous contamination and to maintain a moist atmosphere around the seedlings. The latter at first looked normal, and there was no sign of disease. Three weeks after sowing two of the seedlings showed typical pasmo lesions on the cotyledons. Three days later two other seedlings in the pot developed similar lesions, and in due course all the lesions developed pycnidia of *S. linorum*. This is conclusive evidence that infection originated in the seed.

As already shown, pycnidia of *S. linorum* can develop rapidly on infected flax seed when the latter is placed in a moist atmosphere, and it follows that pycnospores from such fructifications would under moist conditions stream on to the cotyledons in those cases where the seed coats are carried above ground and remain attached to the seedlings. A higher percentage of infected seedlings would be expected to occur under field conditions, where growth of the seedlings in the early stages would be much slower than was the case in the glasshouse.

Discussion.

Since it was first observed on the continent of Europe in 1936, the occurrence of the pasmo disease of flax has been remarkable. In the past six years it has been reported in five different countries, the most recent record being that from Ireland. The occurrence of the disease in Ireland on wild flax raises the interesting question as to how this host became infected. The field from which the infected plants were collected has been in pasture for a number of years, so that if the wild host became infected from cultivated flax growing in the same field the disease must have been present here for many years. On the other hand, infection of the wild flax may

have been initiated through the agency of wind blown spores from a diseased crop in the neighbourhood. With regard to this possible source of infection, a selection of oil flax varieties has been grown for a number of years in the vicinity of the pasture-field in question, the seed being imported mainly from the U.S.A. An examination of these varieties was made in 1945 in the month of June, but no trace of pasmo was found, nor has any disease resembling pasmo ever been noted on them. Nevertheless, the suspicion that the disease originated from such imported seed cannot be dismissed.

A third possibility, however, presents itself—namely that the wild flax may have become infected through linseed cake used as cattle food on this land. This is a factor which must now be considered in view of the information which has come to light as a result of the present work. It is interesting to note in this connexion also that Dr. Foister, of Edinburgh, in correspondence with one of us records the finding of the pycnidia of *S. linorum* in 1941 on flax seed which had reached Scotland for conversion into cattle food and oil.

As to the distribution of the pasmo disease in Ireland, a careful survey of fibre flax crops was made in 1945 in all the flax-growing areas of the twenty-six counties. This established the fact that the disease is by no means widespread here, as it was found in only two cases throughout the year. The first case was from a farm in Co. Cork on seedlings which were forwarded to this laboratory in the month of May. Incidentally, it should be stated that the farm where this outbreak of the disease occurred was approximately 35 miles distant in a straight line from the farm where diseased plants of *L. angustifolium* were found in 1944. Moreover, a search of the farm and neighbourhood failed to reveal any infected plants of *L. angustifolium*.

The occurrence of the disease under such circumstances suggests that the parasite was probably seed-borne. The seed from which this crop was grown was, however, produced in England, and, as the disease is not known to occur there, the origin of the disease in this case is difficult to trace. Nattrass (9) in Kenya observed a severe outbreak of pasmo under similar circumstances, and was driven to the conclusion that the fungus could only have gained entry to that country on flax seed, which he assumed had come from America.

Regarding the occurrence of the second case of a crop diseased with pasmo the specimens of affected plants only came to hand towards the end of September, 1945. The crop was an oil flax of the variety Redwing grown for feeding purposes. It was raised from seed saved in Co. Kilkenny the previous year. It is interesting to note, however, that the seed which gave rise to the 1944 Kilkenny crop emanated from the Co. Cork farm on which the pasmo-infected plants of *L. angustifolium* originally occurred. This case is a further indication of the dissemination of the parasite through the agency of infected seed, and the results of the present investigation leave little doubt as to the important part which infected seed plays in such dissemination.

The survival of *S. linorum* during the winter is possible by any of the following ways:—

(*a*) As pycnospores on the surface of the seed or as infected detritus amongst improperly cleaned seed samples.

(*b*) By means of diseased stubble, as has been shown by Brentzel (2).

(*c*) By means of the perfect or perithecial stage of the fungus.

(*d*) As pycnidia and mycelium in the walls of the infected seed.

There is as yet no experimental proof that the parasite does survive in the manner suggested in (*a*), and, since seed dressing would be expected to minimise the infection of a crop grown from such seed, its importance as an overwintering method is thereby lessened. As regards (*b*), this is not regarded as of significance in this country, since in the case of fibre flax, at any rate, the crop is pulled leaving no stubble and although crop debris may remain on the ground after pulling this is generally ploughed under in subsequent tillage operations. Furthermore, due to the rotation practised here, flax is very rarely sown for two consecutive years in the same soil. In the case of (*c*), perithecia of *S. linorum* have not yet been seen by us, but, since the amount of diseased material which we have examined has been relatively scanty, the possibility of the formation of perithecia on diseased tissue, particularly of flax grown for oil or feeding, cannot be ignored.

The writers are, however, of the opinion that *S. linorum* overwinters and is disseminated, in this country at least, mainly as mycelium and pycnidia in the walls of flax seed from diseased crops. Support is lent to this view by the difficulty which has been experienced elsewhere in controlling the disease by means of surface seed dressings.

Seed infection of flax originating from the placenta has been described by Lafferty (6) in the case of *Polyspora lini*, the cause of Browning and Stem Break of that crop. It differs, however, from the method of infection described here for *S. linorum* in that the fungus in the former case penetrates the wall of the seed boll and thus reaches the placenta, while in the latter the parasite reaches the placenta from the pedicel.

In view of the fact that the pasmo disease appears to have been introduced into Ireland on oil flax or on oil flax products, and also since the prolonged period of growth of this crop beyond that of fibre flax would render it more liable to severe infection, it is considered that further observations on the occurrence of pasmo on oil flax are greatly to be desired. It is hoped to carry out during the 1946 season a further survey of diseases occurring on fibre flax.

ACKNOWLEDGMENT.

The best thanks of the Authors are due to Dr. Phyllis E. M. Clinch, who prepared the serial sections of diseased seeds.

SUMMARY.

1.— The occurrence throughout the world of the pasmo disease of flax and its distribution in Ireland are discussed.

2.—The symptoms of the disease are described on seedlings and on maturing plants. The fungus infects cotyledons, stems, leaves, sepals, pedicels, and internal parts of the boll. The occurrence of a resistant stage in the growth of the flax plant is emphasised.

3.—The causal fungus is described in pure culture, and the union of germinating pycnospores is noted.

4.—Seed infection by the formation of mycelium and pycnidia in the seed coat is demonstrated for the first time. The position of the pycnidium in the layers of the seed coat is described.

5.—The manner in which *S. linorum* may overwinter is discussed, and the conclusion is reached that the pasmo disease of flax is initiated chiefly through the agency of mycelium and pycnidia in the seed coat.

EXPLANATION OF PLATES.

Plate 2.

Fig. 1.—Cotyledons of flax seedlings showing pasmo symptoms produced by spraying with a suspension of pycnospores of *S. linorum*. Slightly enlarged.

Fig. 2.—Development of pycnidia of *S. linorum* on the dead cotyledon of a flax seedling which has been sprayed with a suspension of pycnospores of the fungus. × 3.

Fig. 3.—Portion of flax plant at the stage of boll formation showing the blighted effect produced by infection with *S. linorum*. Slightly reduced.

Fig. 4.—Enlargement of a leaf lesion from Fig. 3. This shows the bleaching of the central portion of the lesion and the production of pycnidia. × 6.

Fig. 5.—Portion of a flax plant at the same stage of growth as Fig. 3 showing the elliptical lesions on the stem. Slightly reduced.

Plate 3.

Fig. 6.—Flax boll infected by *S. linorum* showing the development of pycnidia on the sepals and decay of the pedical at its junction with the boll × 3.

Fig. 7.—Interior of flax boll as shown in Fig. 6. Note the elongated dried spore horns arising from pycnidia embedded in the placenta. × 6.

Fig. 8.—Portion of stem of flax plant diseased with pasmo showing the spore horns arising from pycnidia in the stem. × 3.

Fig. 9.—Formation of pycnidia on the coat of a flax seed derived from a boll having the pasmo disease. × 12.

Fig. 10.—Pycnospores of *S. linorum* exuded in a tendril from one of the pycnidia shown in Fig 9 after the seed had been incubated in a moist atmosphere. × 55.

Fig. 11.—Pycnospores of *S. linorum* obtained from a pycnidium on the seed coat. × 350.

Plate 4.

Fig. 12.—Germinated pycnospores of *S. linorum* showing anastomosis of the germ tubes. × 200.

Fig. 13.—Chlamydospores which have arisen from pycnospores of *S. linorum*. Note the increase in width of the former over the latter. × 300.

Fig. 14.—Single spore colony of *S. linorum* on potato glucose agar. Note the exudations of pycnospores from the pycnidia in the medium. × 3.

Fig. 15.—Section through the dissepiment of a pasmo-infected flax boll showing pycnidia and contained pycnospores. × 150.

Fig. 16.—Section through a pycnidium of *S. linorum* in the coat of a flax seed. Note the origin of the pycnidium as a stroma between the round cells and the fibrous layer. × 150.

Fig. 17.—Surface view of the dissepiment a section of which is shown in Fig. 15. The dark circles are the pycnidia embedded in the tissues. × 50.

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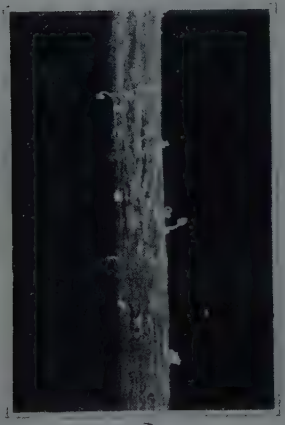

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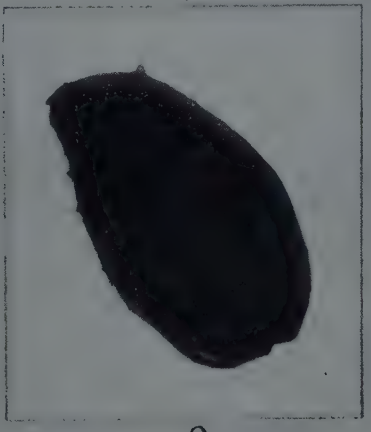

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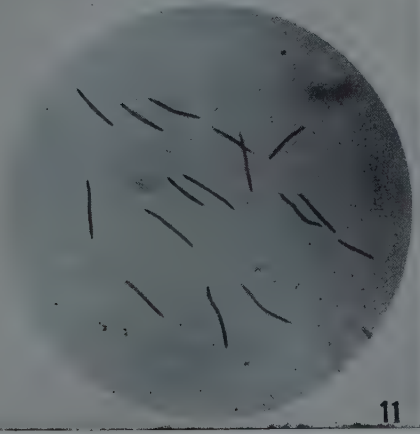

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No. 11.

A STUDY OF *SEPTORIA OXYSPORA* PENZ. & SACC. ISOLATED FROM DISEASED BARLEY.

By

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(PLATES 5, 6, AND 7.)

INTRODUCTION.

THE present paper deals with a disease of barley which was not observed in Ireland until the year 1931. It was recognised as a new disease in this country at that time, and as it appeared sufficiently serious to give rise to some apprehension a considerable amount of time was spent on the investigation of the fungus. The results of this work were not then published, the occurrence of the malady being simply reported as a new disease of barley associated with a species of *Septoria*, a note to this effect appearing in the Second Annual Report of the Minister for Agriculture 1932-33, Appendices page 6.

Since 1931 a careful lookout for this particular disease has been kept each year, but it was not again seen on barley until July 1945, when my colleague Mr. J. B. Loughnane collected some diseased plants in Co. Cork. An examination of these specimens showed the same characteristic Septoria to be present as occurred at Glasnevin 14 years previously. In this case, however, the Septoria was not alone, but occurred along with another fungus, *Rhynchosporium Secalis* on the affected foliage. Subsequent to some further investigation the Septoria was identified as a strain of *Septoria oxyspora*, and as there does not appear to be any previous account of the behaviour of this organism in pure culture, it was finally decided to publish the results originally obtained as a contribution to our general knowledge of this group.

HISTORY OF THE DISEASE AT GLASNEVIN.

The disease occurred on a strain of Spratt-Archer barley. This particular selection was made at one of the Continental State Experiment Stations, and was introduced in the spring of 1931 by the Plant Breeding Division, Albert Agricultural College, Glasnevin. Incidentally the grain was treated with a dry dust preparation before sowing.

The attack was first noticed as a dark brown spotting of the foliage on the tops of a number of plants towards the middle of June 1931. When first seen only a few plants confined to an area of three square feet showed the spotting, and no particular attention was then paid to it. By the 1st July, however, the diseased plants attracted attention from a distance of twenty yards. All round the original point where spotting first appeared the top leaves of the plants were quite yellow at their tips, the discoloration varying from an inch in length on some plants to four or five inches on the leaves of others, with the result that over an area of some square yards the whole ends of the upper foliage appeared as if scorched by fire. On closer examination it was found that on the yellowish patches on the leaves innumerable small spots with a greyish centre surrounded by a purplish border occurred, and further back on the green tissue other similar spots were present. Individual spots varied in size from 1—3mm. in length and were generally elongated in the long direction of the leaf. Very small spots were entirely brown or dark purple in colour. The flag leaf suffered worst (Fig. 1 Plate 5). In many cases this leaf had only a narrow band of green tissue remaining, extending from the base half-way up the lamina, all the rest being chlorotic and practically dead. Although the upper foliage was that most severely attacked, diseased spots could also be found on the lower leaves, mainly on their tips and margins. It was obvious that the killing of the foliage was due chiefly to the numerous spots which were coalescing, long narrow strips 2-3 cm. in length being killed outright. The auricles and bases of the leaves were not attacked until the remainder of the leaf was practically dead. Every plant in the plot, which covered 24 square yards, showed some of the disease on its foliage. The general appearance of the disease on these barley plants was not unlike that produced by Celery Leaf Spot on celery plants during a severe outbreak of the latter disease in September or October.

No trace of similar spotting could be found on any of the adjacent plots of barley, which comprised fifteen foreign varieties, the sources of these being three European countries, Canada, and The United States. In addition to this foreign collection there were several native varieties and hybrids. As the outbreak and general symptoms did not appear to be identical with any of the usual barley diseases, and as it was evident that the trouble was spreading rapidly, following a consultation with Mr. Caffrey, Head of the Plant Breeding Division, it was decided to destroy the entire plot. The barley was accordingly cut and destroyed. Previous to this a few plants had been potted up for investigation purposes, and the fungus had already been isolated in pure culture from typical diseased specimens.

The plot of barley which was cut on 2nd July sprouted up again from the base, and the same disease was found on the young foliage towards the end of July and again in September. The attack on this foliage always remained slight.

Investigation of the Disease.

When affected leaves are examined closely it is seen that the dead yellowish areas and the grey centre of the spots are studded with small pycnida (Fig. 2 Plate 6). These are just visible to the naked eye as small black dots, and occur in straight rows owing to the arrangement of stomata in barley leaves. Each pycnidium occupies a sub-stomatal cavity, and on the bleached greyish portion of the diseased

area almost every stoma may have one below it, so that pycnidia are often touching each other in the rows. Owing to their position a line of pycnidia is often found on each side of a leaf vein, two rows occurring between each pair of veins. The fructifications occur on both sides of the leaf, but they are more abundant on the upper surface. Pycnidia are slightly flattened on the top, and have their greatest diameter parallel to the vascular bundles (Fig. 3 Plate 6). They vary in size from 76μ x 72μ to 119μ x 78μ, and their breadth is more constant than their length, the average width being 77μ. These measurements are from fresh material.

Examination with a compound microscope shows more or less of a pattern on the pycnidial wall, which is pseudo-parenchymatous, brownish to almost black. This wall is thin and composed of dark short-jointed hyphae interwoven together, it is usually about 6μ in thickness, and seldom exceeds 9 or 10μ. It is frequently best developed and darkest in colour around the ostiole. The latter coincides with the stomatal opening, the guard cells of which are so affected that they are rendered incapable of functioning, the aperture being never closed but fully distended, thus providing escape for the spores to the outside. When a small piece of leaf containing pycnidia is placed in water on a slide a mass of spores exudes through the stomatal opening, and in material fresh from the field the spores may be found either projecting from the stomata or a whole mass of them can be found outside the aperture.

The pycnospores are thin-walled, hyaline, typically more or less crescent shaped, (Figs. 4 & 5, Plate 6), they are slightly blunt at their heel end and bluntly pointed at their apex. While the majority of the spores conform to this type, some are shaped like boomerangs, and occasional ones like hockey sticks. Spores vary in size from 15—22μ long by 2—4μ wide, with an average length of 18μ and breadth of 3μ. The majority of the spores are unicellular at the time of emergence, but some are to be found with one septum (Fig. 5, Plate 6), or occasionally two cross walls. Spores left in water overnight swell up, and some of them develop cross walls, the number of septa being 1—3. Germination can take place at any part of the spore, and may occur at two or more points at the same time; many spores germinate without developing cross walls. Although some germination takes place in distilled water it is much more abundant in weak sugar solutions or in barley extract agar in 24 hours. Spore germination is somewhat variable, the most usual method is for a small protuberance to appear on the spore wall, the outgrowth developing into a hyphal thread with numerous septa. This hypha may give rise to other similar hyphae, or frequently in nutrient solutions, and especially in barley extract agar, it commences to bud off conidia exactly similar to the pycnospores. Such conidia may be borne directly on the side of the hypha, or a small projection may arise on the hyphal thread, from which conidia are abstricted, the older conidia being pushed aside by the younger (Fig. 11, Plate 7). These side growths may be very numerous; they differ in size from a mere swelling on the side of the cell to one, two or more cells, exactly like those of the parent hypha, and at the end of these subsidiary growths conidia are produced. After spore germination the first-formed hypha may develop thick walls, and from this thick-walled closely septate hypha a thin branch arises, conidia becoming abstricted from its free end (Fig. 13, Plate 7). Very often such thin-walled branches are about 22μ long by 3μ wide, consist of a single cell, and resemble a conidium which has remained attached to the parent hypha.

At the end of 48 hours after germination in barley extract agar the hyphal threads vary in thickness from 3 to 12μ, the thicker strands being formed mainly of short thick-walled cells, the length of which seldom exceeds 11μ and very often is only about 6μ. Pycnospores may also germinate by a process of budding, producing one or more similar spores, (Fig. 10, Plate 7). These methods of conidial production are typical of growth on barley extract agar, and at the end of 48 to 72 hours after germination of the pycnospores all trace of mycelium is hidden under the mass of conidia produced. The only difference between conidia and pycnospores is that the former are not so curved as the latter, but even in the case of pycnospores the difference in curvature is considerable. At the time conidia are abstricted, they are from 18 to 22μ long, but in nutrient solutions they quickly swell up and may reach a size of 30μ by 4μ, though about 24μ is the average length. The conidia behave similarly to pycnospores ; a few develop cross walls before germination, but the majority germinate without the formation of septa, and others bud off similar conidia direct. Development of conidia may be easily followed under the microscope by mounting a small portion of thick-walled closely septate hypha in barley extract in a hanging drop (Figs. 13—17, Plate 7).

Single pycnospore isolations of the fungus were made from diseased field material on potato glucose agar, and as all separate isolations appeared identical in pure culture one monospore isolation was retained, and all the others discarded. From this single spore isolation sub-cultures were made to different media and the behaviour of the fungus studied. Other cultures derived from the same source were used for inoculating barley plants, and in every case the same diseased condition of the host resulted and the parasite was recovered without change.

Production of the Disease in Barley Plants.

The methods of inoculation followed were to raise barley seedlings in pots, and when the plants were over two inches high to inoculate, either by spraying with conidial suspensions in water or by placing a little of the medium containing the fungus direct on the leaves, the latter being unwounded. Controls were sprayed with tap water at the same time. After inoculation the pots with their appropriate controls were covered with bell-jars for 48 hours. Both methods of inoculation were successful, and spraying with conidial suspensions in water was the only one used in later work. In the first inoculation performed in September 1931 the pots were kept out of doors, and the disease only developed to a very slight extent. These plants remained outside throughout the winter with only an occasional diseased spot visible on the leaves until near the end of January 1932. By this time the plants were twenty inches high, and the disease then assumed a much more severe form on the upper leaves. In subsequent inoculations during the winter of 1931-32, the plants after spraying with spore suspensions were covered for eight days and were kept in an unheated greenhouse. In these latter cases every plant developed the disease, and it was more typical of what appeared in the field. It should be stated here, that in every case where young plants became diseased after inoculation they afterwards grew out of the disease to a great extent, and then when they were coming into ear the disease became virulent on the upper foliage. This is the period when the plant is most susceptible to the disease, and the attack was always worst

on the upper leaves and at their tips and margins. Five separate inoculations on barley plants were carried out in the unheated greenhouse during the winter of 1931-32, and the disease was produced on each occasion, the fungus being recovered without change.

Following infection in the greenhouse with conidial suspensions the first signs of disease appeared on the 12th day, when numerous pale yellow spots were seen towards the tips and margins of the leaves. The middle of these spots appeared slightly depressed, and five or six days after they became visible a brown margin developed around each of them ; the centre of the spot soon became grey and bleached in appearance, and pycnidia developed. The pallid spots on the foliage may be easily detected in an early stage by holding up the leaf between the observer and the light. They vary in size from less than 1mm. to 3mm. in diameter; usually when the diameter of the spot reaches a length of 1mm. the brown margin appears. Where individual diseased spots are isolated the line of demarcation between diseased and healthy tissue is fairly distinct, but where the spots are closer together this line is not so definite; the dark brown border fades into yellowish-green, the spots rapidly coalesce, and soon the whole area becomes grey and bleached, and pycnidia develop. Microscopical examination of the pale spots on the 12th day following infection showed a septate mycelium 3—5 μ wide in the leaf tissues. The fungus here is fairly uniform in thickness, mainly intercellular at first, but very soon the cells are invaded and the parasite is both inter- and intra-cellular. Hyphae invariably make their way to the sub-stomatal cavities, where numerous septa appear in them, and they become interwoven together forming a small weft of mycelium at the bottom of the air space, and all stages of pycnidial development could be found. The pycnidia are quite grey at first and invisible to the naked eye, even with the aid of a good pocket lens, but they can be detected with the compound microscope, and even in this early stage of development the whole cavity of the air chamber is often filled with spores. The ostiole is directly under the stoma, and the hyphae around it become brown as do also the basal layers of the pycnidium. Finally the whole structure darkens and can then be seen with the naked eye as a small black speck. Formation of pycnidia may be detected as early as ten or twelve days after inoculation, and perfect fructifications may appear as soon as sixteen days, but it is usually about three weeks before they become visible.

Two inoculations were carried out in the open field during the month of May 1932. In one case a number of plants in a plot of barley were sprayed with a conidial suspension and covered with bell-jars for five days. Typical infection occurred and pycnidia were found sporulating twelve days later. In the second experiment a pot containing diseased plants was placed in the middle of an isolated plot of barley and the disease allowed to spread naturally. The disease was found on neighbouring plants twenty-four days after this pot was placed in position. The weather was humid at this time and favourable to the spread of the disease, which appeared worst on the tips and margins of the foliage. Three or more inches of the leaf tip became completely yellow, this soon bleached in appearance and pycnidia developed in rows. The weather then became warm and dry, and no further spread occurred until the end of July, when there was a spasmodic outbreak during showery weather. This attack, however, remained slight, and no serious development of

disease took place. Controls throughout remained healthy, and no trace of any similar disease could be found on any of the barley plots elsewhere on the farm in 1932.

Attempts were made during the spring of that year to inoculate growing plants of the following: Cocksfoot, Timothy, Oats, Rye, and Wheat, but in all cases the disease failed to go over, although barley plants were readily infected at the same time.

Cultural Characters.

The fungus grows well on a number of artificial media, but the development of mycelium is scanty on the majority of them, and in some cases almost suppressed, the organism developing by conidia budding in more or less of a yeast-like manner.

Cultures on the various media were kept and examined over a period of eighteen months, but pycnidial development could not be induced on any artificial medium. Growth on slants in all cases was better than on poured plates ; on the latter the colonies enlarge very slowly and soon cease growth unless there is a very deep layer of the medium. The following is a description of the growth of the fungus on slants incubated at 20°C:—

Potato Agar.—Growth slight, bacteria-like on surface of slants, colonies less than 1 cm. in diameter at end of two weeks. Mycelium chiefly on surface or slightly submerged. Hyphae much gnarled and twisted, slightly dark in colour, varying in thickness from 2 to 6μ. Thicker branches covered with many short side growths which all produce conidia. Surface of the slants dry in appearance at end of fifty days, no aerial growth and no development of colour in medium. A number of very small sclerotia present but these are never numerous even on cultures twelve months old.

Oat Extract Agar.—There is very good development of mycelium which is entirely submerged, surface of slants dull grey, flat. Hyphae of a fairly uniform thickness 3-6μ broad. A few swollen cells of mycelial threads seen but very scarce, conidia produced in moderate numbers. There is no darkening of hyphae, as occurs on other media. On cultures six months old the mycelium was all through the medium although the appearance of tubes did not suggest this. A few sclerotia formed on surface of slant but they were scanty.

Dox's Medium.—Growth extremely poor, consisting of abnormal submerged mycelium, hyphae much gnarled, twisted and swollen, forming a growth like a flattened lichen. No conidia or sclerotia produced.

Barley Extract Agar.—At the end of fourteen days colonies over 2 cm. in diameter, moist to slimy on the surface like bacterial growths, colour dirty pink in middle, growth consists chiefly of budding conidia. Colonies reach a diameter of 5 to 5½ cm. at the end of one month, but general appearance does not change. This is the ideal medium for conidial production, and though a few minute sclerotia develop on old cultures their number is relatively small. Cultures over nine months old continue to produce conidia. A dirty pink colour appears early in the middle of the growth, but the colour never spreads to any extent nor becomes intense. In cultures under one month old hyphae which are present are hyaline and have their cell walls regular, only very rare cells being swollen. In older cultures the hyphal threads vary in thickness from 2-6μ. The same hypha may be

quite thin, then part of it may become twice as thick with correspondingly thick cell walls, and at the end of a number of swollen cells the hypha may return to its thin-walled condition again. Hyphal threads also occur which have alternately thick and thin cells.

Ground Quaker Oat Agar.—Growth on this medium is perhaps the best of any consisting chiefly of conidia at first, but plenty of submerged mycelium is produced, which grows just below the surface of the slant. Colonies reach a diameter of 2 cm. in seven days, and at the end of three weeks cover most of the slant. Middle of growth slimy, flat, dull grey to whitish at margins. Sclerotia form rapidly on surface of slants, the majority of these have a diameter under $\frac{1}{2}$mm. but some over 1 mm. The mycelium is uniform in thickness with a width of 4-5μ, and in the early stages of its growth very few or no short swollen cells occur. A tough "leathery" crust is formed on the surface of slants, and as the culture ages the mycelium darkens at the surface, and the crust becomes raised up into folds with innumerable sclerotia dotted over it. The bottoms of these folds often remain moist and slimy in appearance, and conidia continue to be produced on such spots for a long time. On cultures over nine months old a few dirty white pustules of the fungus may be present on the surface, the amount of aerial mycelium is always very small and only the surface of the medium blackens. Sclerotia may develop on one section of plates while other sections are devoid of these bodies ; cultures raised from such sectors behave like original cultures on slants.

Potato Glucose Agar (glucose 2%).—Growth on this medium is very similar to that on ground Quaker Oat agar. In fourteen days colonies reach a diameter of 2 cm. with a trace of sclerotial formation on their surface. Growth slimy, pink to deep salmon pink in colour, with numerous conidia and sclerotia at the end of 3 weeks (Fig. 6, Plate 6). Mycelium practically all submerged with a uniform thickness of 4-5μ. The mycelium soon darkens and a tough crust forms on the surface of the slants which rises up in ridges. Growth covers most of the slant at the end of one month and conidial and sclerotial formation go on side by side, the former generally in the hollows which are moist and slimy looking. In some tubes a few whitish pustules of aerial mycelium appear, but their growth is always scanty. In cultures over nine months old the whole surface of the slant appears black owing to the innumerable sclerotia present, and also to the darkening of the submerged mycelium.

Potato Glucose Agar (glucose 4%).—At this concentration of glucose, growth is very similar to above but there is a more intense development of colour, and the conidia produced are often larger than those formed on the 2 per cent. glucose or any other medium. The difference in size of conidia is most marked in tubes held at 4-5°C., (Fig. 12, Plate 7). At this temperature all sizes of conidia may be found from 15 up to 41μ long by 4-5μ wide: cultures on the same medium at 20°C. show conidia only slightly larger than normal. At the lower temperature on 4 per cent. glucose agar, the spores apparently go on absorbing nutriment and increasing in size without germination and generally without the development of cross walls.

Conidia produced on barley extract agar, ground Quaker Oat agar, and potato glucose agar 2% are fairly regular in size, being 22-24μ long by 3.5-4μ wide. These are measurements of spores found in cultures ; as mentioned previously, when first

formed the spores are usually about 18μ long, but they rapidly enlarge in nutrient solutions.

Sterilized Barley Leaves.—Leaves of barley plants sterilized in tubes and inoculated with the fungus from pure cultures developed a white fluffy scattered aerial growth of mycelium over their surface. Interspersed amongst this mycelium sclerotia were found, all on the surface of the leaf. In a few cases small groups of hyphae produced conidia similar to those in pure culture, and this continued up to the end of sixty-eight days. The leaf tissues were permeated with mycelium of a thickness from 2.5 to 5μ having an average width of 3μ. Only sclerotia developed on the first batch of leaves inoculated, but some pycnidial formation was induced in subsequent lots by placing a small piece of cotton wool saturated with water in the bottom of the tubes previous to sterilizing. The pycnidia formed in rows in the leaf tissue, as in the growing plant, and produced pycnospores. Immature fructifications were present at the end of twenty-one days after inoculation, but it was not until sixty-eight days had elapsed that pycnospores were found, and only a few perfect pycnidia were then observed, the others being immature or seemingly arrested in their development. Many of the tubes of inoculated sterilized leaves contained no conidia, mycelium and sclerotia only being present.

The Sclerotia.—The shape of these as in all similar bodies varies somewhat, the majority are round to oval in section, others occur which are thin flattened structures two or three times as long as they are broad. On all media, even when produced in large numbers, the individual sclerotium remains small, usually having its greatest diameter about 300μ, and seldom exceeding 500μ in those cases where it is oblong or flattened. Sections 10μ in thickness (Fig. 7, Plate 6) show them to be true sclerotia with a distinct outer rind, sometimes unequally developed, composed of thick-walled dark brown hyphae surrounding a closely packed mass of mycelium which is thin-walled and hyaline. The outer layer may vary from 12 to 60μ in thickness. In cultures larger black bodies of 1 mm. or more in size occur. These when sectioned are seen to be made up of two or more (up to 8) single sclerotia which during development have grown together. In these aggregates the adjacent corners of the sclerotia are frequently very much thickened.

Cultures from single Conidia and from Sclerotia.—During the investigation of the disease cultures derived from single conidia, produced on barley extract agar, and also from single sclerotia (the latter from cultures over 12 months old) were raised and studied alongside those derived from the original single pycnospore isolation; but no difference could be detected between them, and they all behaved alike when grown under similar conditions.

Temperature Relations of the Fungus.—Between 12° and 20°C. growth was fairly even with the optimum around 20°C. The maximum for growth was 31°C., but above 27° growth was abnormal, the cultures becoming very pink or coffee-coloured, and the hyphae much gnarled and twisted with scarcely any spread on the slants, although conidia were produced at 31°C. The minimum for growth was not determined, but growth can take place at 4°C., as fresh transfers of pure cultures placed at this temperature grew and increased slowly, eventually covering the whole surface of the slants. The thermal death point of the fungus is between 45° and 48°C. The majority of young vigorous cultures under two weeks

old and also of older cultures up to an age of 289 days do not survive immersion for ten minutes in a water bath at 45-46°C., but on two occasions one each of a pair of cultures heated at 47°C. for ten minutes grew. In one case the treated culture was sixty-four days old, and growth did not commence until ten days after the transfer was made, while in the second instance growth of the transfer was just visible one month after treatment. In both these cases of delayed development the mycelium which eventually grew was sterile, but on transferring again to fresh tubes of potato glucose agar or of barley extract agar the characteristic conidia of the fungus appeared.

Longevity of Pycnospores.—Diseased barley leaves collected early in July 1931 were kept in an envelope in a locker in the laboratory, and pycnospores obtained from these leaves were tested for germination periodically. Small pieces of foliage containing pycnidia were placed in water and in weak solutions of sugar and teased out with needles to enable the spores to escape, the prepared slides being incubated at 20°C. Germination was good up to the end of four months, medium at seven months, and dropped rapidly after this. At the end of nine months many of the spores swelled up in the liquids but comparatively few germinated ; at the termination of eleven months the germination was only 3 per cent., and this dropped to a very odd spore after fourteen months. Germination of an occasional spore was still obtained at the end of sixteen months, but scarcely any of the remainder appeared swollen.

Discussion.

Several members of the genus Septoria are known to attack wheat, oats, rye, barley and various grasses. Amongst them *Septoria Passerinii* Sacc.—which has large septate spores—is the one most commonly reported as occurring on barley throughout the world. Another species, however, has been recorded on this host, viz. *Septoria culmifidia* Korst (3). When the disease first occurred at Glasnevin in 1931 and the fungus had been isolated and studied in pure culture, its development on different media did not agree with that of any known cultured Septoria, and unfortunately cultures of *S. culmifida* for comparison were not obtainable at any of the following institutions:—Imperial Mycological Institute ; Lister Institute (National Collection of Type Cultures), or Centraalbureau voor Schilmmelcultures Baarn, Holland.

On the recurrence of the disease in 1945 specimens of affected foliage were sent to Kew, and R. W. G. Dennis, who replied to the enquiry, suggested that the fungus was most likely a race of *Septoria oxyspora* Penz. & Sacc., and further suggested that cross inoculations to grasses might be carried out. This had already been done with entirely negative results. Dennis also kindly forwarded a specimen of *S. oxyspora* on a leaf of *Phleum pratense*. From a comparison of this material with diseased barley foliage no morphological difference could be detected between the pycnidia or pycnospores. It is therefore concluded that the fungus responsible for the disease on barley in Ireland is a strain of *Septoria oxyspora* Penz. & Sacc.

So far as the author is aware, there is no previous record of the occurrence of *S. oxyspora* on barley in the British Isles, although the fungus is fairly common on grasses (2). It has, however, been recorded on barley in Norway (3) under the name *Septoria culmifida*. Jorstard (4) reports that in the north of Norway this Septoria often occurs in profusion on barley together with *Rhynchosporium Secalis*, the Maskin variety being particularly susceptible. Grove (1) was apparently the first to record *S. oxyspora* from the British Isles, the fungus occurring on *Arundo Donax*. Later, Miss Sampson (5) reported it under the name *S. culmifida* on the following grasses at Aberystywth, *Alopecurus pratensis*, *Poa trivialis*, *Dactylis glomerata*, *Phleum pratense* and *Arrhenatherum elatius*. In a more recent publication by Miss Sampson and J. H. Western (6) the fungus on these grasses is referred to as *S. oxyspora*, the former name *S. culmifida* evidently being regarded as synonymous. Grove (2) in his description of the fungus states that frequently the spores are shaped like a boomerang, while Miss Sampson found them to be typically crescent shaped (6). In the barley fungus examined by the author both types occur, but the crescentic shape prodominates.

The formation of conidia in pure culture seems to be common to a number of Septorias. Weber (7 & 8) who made a critical study of *Septoria* diseases of cereals and grasses found that conidial production occurred freely in the majority of the eight species studied, but all of them eventually produced pycnidia, and some of them perithecia also. Weber makes no mention of sclerotial formation in pure culture in any of the species investigated by him. *Septoria oxyspora* in pure culture formed conidia freely, often by a process of budding, but neither pycnidia nor perithecia developed apart from the host. Nevertheless, sclerotial formation was abundant on several media in addition to conidial production.

Although the first outbreak of this disease on barley was rather alarming, so much so that it was considered necessary to destroy the affected plot, the disease is not now regarded as of serious importance. It is probable that the particular strain of barley imported from Denmark in 1931 was very susceptible to the fungus, and this would account for its virulent development. From the evidence available it would appear as if the strain of *Septoria oxyspora* Penz. & Sacc. occurring on barley is more or less specialized on this host.

The author wishes to express his thanks for information kindly supplied by the following:—R. W. G. Dennis, Kew ; Miss Kathleen Sampson, late of University College, Aberystwyth, Wales ; and Dr. J. H. Western, The University, Manchester. I am also indebted to my colleague Dr. Phyllis E. M. Clinch for the preparation of stained spores illustrated in Figs. 4 and 5 and for assistance with the drawings.

Summary.

A disease of barley which first appeared on plants grown from imported grain in 1931 was again found on barley plants in 1945.

The causal fungus was isolated, studied in pure culture, inoculated to barley plants and the disease reproduced. It is believed to be a strain of *Septoria oxyspora* Penz. & Sacc.

Conidia are freely produced in pure culture on several media: This development is often accompanied by the formation of sclerotia, and in this respect the fungus appears to differ from any known Septoria which has been cultured from the small cereals or grasses.

The disease on barley is of no economic importance at the present time in this country.

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Explanation of Plates.

Plate 5

Fig. 1. Severe infection of flag leaf of barley by *Septoria oxyspora :* typical appearance of the disease as it occurred in 1931.

Plate 6.

Figs. 2, 3, 4, 5 and 7 photomicrographs.

Fig. 2. Diseased portion of barley leaf showing linear arrangement of pycnidia of *Septoria oxyspora* below stoma. Section cleared in Berlese's fluid. x 35.

Fig. 3. Longitudinal section through two pycnidia showing variation in size and general structure. x 275.

Fig. 4. Group of pycnospores. Stained with Methyl Blau. x 190.

Fig. 5. A single pycnospore showing a cross wall. x 360.

Fig. 6. Culture of *Septoria oxyspora* three weeks old on potato glucose agar showing numerous sclerotia.

Fig. 7. Section of sclerotium from pure culture, 10μ thick. x 110.

Plate 7.

All Figs. on this plate camera lucida drawings and all to magnification of 840.

Fig. 8. Germination of pycnospores in 5 per cent. cane sugar solution at end of 24 hours.

Fig. 9. Appearance of germinated pycnospores in 5 per cent. cane sugar solution at end of 96 hours.

Fig. 10. A pycnospore budding off conidia direct in barley extract agar.

Fig. 11. Thick walled, closely septate hypha producing conidia in barley extract agar.

Fig. 12. Conidia in 4 per cent. potato glucose agar at end of 14 days held at temperature of 4°-5°C.

Figs. 13-17. Successive stages in development of conidium in hanging drop of barley extract agar.

Fig. 13. Appearance at 2.0 p.m. attached spore 15.5μ long.

Fig. 14. 3.50 p.m. Appearance of new spore.

Fig. 15. 4.20 p.m. New spore 11μ in length.

Fig. 16. 4.30 p.m. Both spores still attached.

Fig. 17. 4.40 p.m. Older spore abstricted.

Photo by *G. H. McLean*

Fig. 1

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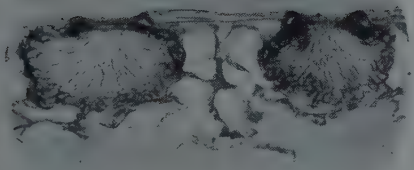

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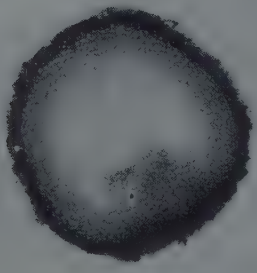

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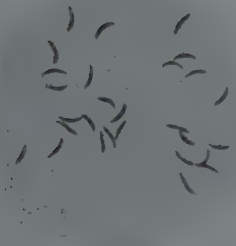

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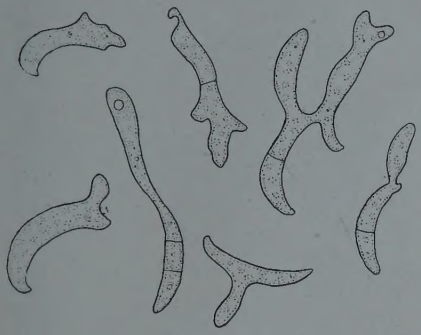

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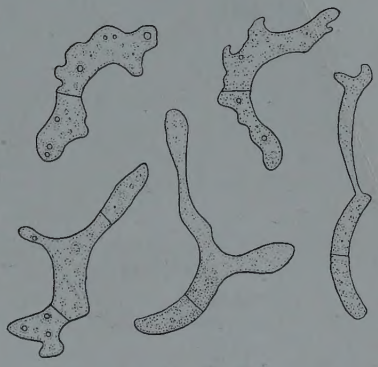

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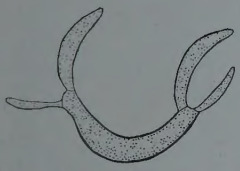

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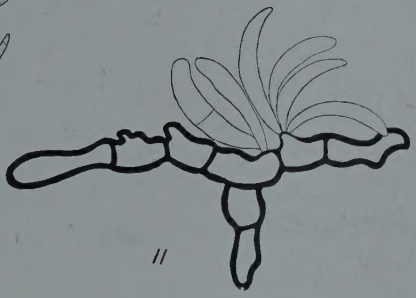

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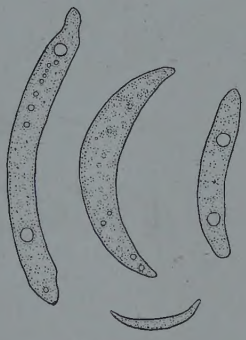

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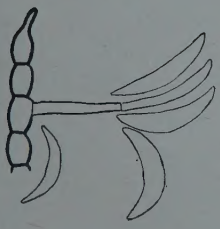

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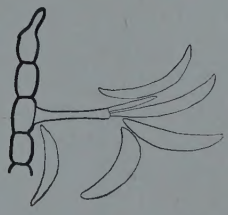

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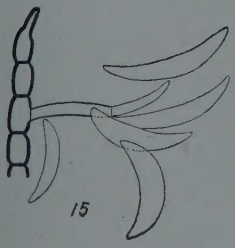

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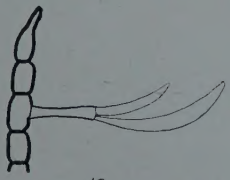

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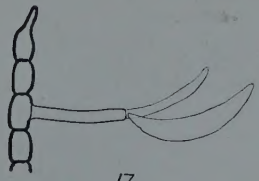

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